

FINAL REPORT

i. Title Page

COMPETITION AND EGG-HATCH INHIBITION AMONG TIRE-BREEDING MOSQUITOES

NOVEMBER 13, 1993

Sponsoring agency: FDACS, Bureau of Entomology and Pest Control

Principal Investigator:

Todd P. Livdahl
Department of Biology
Clark University
950 Main Street
Worcester, Massachusetts 01610
Telephone: 508 793-7514

ii. Summary

Project Title:

Competition and egg-hatch inhibition among tire-breeding mosquitoes

Principal Investigator and author:

Todd P. Livdahl
Department of Biology
Clark University
950 Main Street
Worcester, Massachusetts 01610
Telephone: 508 793-7514

This project focused on larval competition and larval inhibition of egg hatch between container-breeding mosquitoes that may influence the eventual outcome of two recent species introductions, *Aedes albopictus* from Asia and *Aedes bahamensis* from the Bahamas. Interactions of these species with resident species were assessed within discarded tires. Tires in southern states, once dominated by *Ae. aegypti*, have recently been occupied by *Ae. albopictus* throughout the Southeast, and also by *Ae. bahamensis* in southern Florida.

The relevance of competition to the invasion of Florida by *Ae. albopictus* was established by sampling larvae in tires in urban and rural sites within eight counties (Duval, Alachua, St. John's, Volusia, Indian River, Martin, Okeechobee and Broward) in Florida that have been exposed to *Ae. albopictus* for different periods. Competition, if important to natural populations, should have reduced the abundance of *Ae. aegypti* to a greater degree in areas occupied the longest by *Ae. albopictus*, and an inverse correlation should also exist between *Ae. aegypti* abundance and the abundance of *Ae. albopictus*. This pattern was found in the sampling study: *Ae. aegypti* appeared only in the southern counties of our study; tires in the northern counties, where *Ae. albopictus* was first discovered in Florida, were occupied most frequently by *Ae. albopictus*. We also learned that: 1) the southward advance of *Ae. albopictus* has been more rapid within rural than urban sites; 2) *Ae. albopictus* has now extended its range into Broward County; and 3) *Ae. albopictus* has reached an apparent equilibrium density in the northern

counties. No significant differences in mean larval density were found among rural sites in Duval, Alachua, St. John's, Volusia and Indian River Counties, all of which had significantly higher densities of *Ae. albopictus* than the three more southern counties.

Competition between *Ae. albopictus* and *Ae. aegypti* was examined experimentally by raising larvae of each species at a variety of population density and species combinations in field situations. This experiment produced information necessary for quantitative predictions of the outcome of the *Ae. albopictus* introduction at the population level, with the assumption that competition among larvae is the only major interaction occurring between these species. The results provide clear evidence that: 1) density of larvae within each species is an important factor limiting their success; and 2) neither species was sensitive to differences in density of the other species. That is, competition within each species is likely in natural populations, while larval competition between them is not. The results support a prediction of stable coexistence between these species, which is clearly refuted by our sampling results. We therefore reject the hypothesis that competition has been the force behind the displacement of *Ae. aegypti* by *Ae. albopictus*.

This project included a test of the inhibition of egg hatching by larvae in tire situations. Eggs of *Ae. aegypti*, *Ae. albopictus*, and *Ae. bahamensis* were exposed to four density levels of each species of larvae. Species-specific differences among egg responses to larvae, which may result from differential sensitivity of eggs to larvae or from different larval inhibitory tendencies, were found in an earlier laboratory study but were not apparent in this field investigation. We found that the larvae of these three species do inhibit egg hatch in tires, and that the larvae of all three species had virtually interchangeable effects on egg hatch. In addition, we found similar responses of each species' eggs to the density of larvae, with consistently higher hatch rates for *Ae. bahamensis* at all densities. We conclude that this interaction cannot account for the displacement of *Ae. aegypti* by *Ae. albopictus*, although it may have important effects in combination with other interactions.

Further understanding of the interactions affecting mosquito populations may be crucial to programs aimed at predicting future abundance. Informed predictions will enable mosquito

control programs to focus their efforts where they are most likely to maximize benefits and minimize environmental and public health hazards. These results indicate clearly that the displacement of *Ae. aegypti* by *Ae. albopictus* cannot be explained without more understanding of ecological factors affecting stages other than larvae.

iii. Introduction

The container-breeding mosquito *Ae. albopictus* has become established on the North American continent during the last several years. Signs of the successful colonization of this species were first discovered in Houston in 1985 (Sprenger and Wuithiranyagool 1986), where it had already achieved a dominant position in artificial containers. Since then, *Ae. albopictus* has been found in numerous localities. The Society of Vector Ecologists Newsletter has published reports of *Ae. albopictus* in four midwestern states (Missouri, Illinois, Indiana and Ohio), and in states on both coasts (Delaware, California). The introduction of *Ae. albopictus* has great practical significance to workers in public health and medical entomology, and relates directly to the most basic goals of population biology and community ecology: the understanding of interactions within and between species, especially those that influence distribution and abundance.

The success of *Ae. albopictus* has important potential consequences to public health, because it is a known vector of viral diseases including dengue, and its potential to transmit other viruses has been documented (cited in Hawley et al. 1987). In addition to the potential importation of exotic viral diseases from Asia, the species could become a vector of endemic North American arboviruses, possibly increasing their ranges or incidence. Even if its potential as a vector is not realized, the additional competition imposed on resident mosquito populations could result in an enhancement of their potential to transmit endemic arboviruses. Grimstad and Walker (1991) have shown a two-fold increase in transmission rates stemming from increased susceptibility to infection by LaCrosse virus across mesenteron membranes in nutrient-deprived *Ae. triseriatus* females.

Ae. albopictus has already demonstrated an ability to establish itself quickly in artificial habitats, such as discarded automobile tires. In some areas, such as Louisiana (J. Freier, personal communication), Texas (Sprenger and Wuithiranyagool 1986) and northern Florida (G. O'Meara, unpublished data), *Ae. albopictus* has achieved a dominant position in these aquatic communities, and that dominance was achieved in Texas prior to the first discovery of the species on this continent. There is strong evidence, based on egg diapause responses to

photoperiod and egg resistance to freezing, that *Ae. albopictus* has immigrated from temperate Asian populations (Hawley et al. 1987). Thus, the invading population has arrived with two key adaptations necessary for its successful colonization of much of North America (Nawrocki and Hawley 1987). There is further evidence, based on comparisons of strains derived from North American *Ae. albopictus*, that critical photoperiod for egg diapause and egg freezing tolerance have been adjusted in accord with the length and severity of winters in North America (Pumpuni et al. 1989, Hawley et al. 1989). If variation for these traits is inherited, *Ae. albopictus* will provide a compelling example of the potential speed of natural selection, indicating strong potential for its northward range expansion as a result of adaptation to local climatic conditions.

The experiments within this project examine interspecific competition among larvae of Florida tire-breeding *Aedes*, as well as larval inhibition of egg hatching (Gillett et al. 1977, Livdahl et al. 1984; see Livdahl and Koenekoop 1985 for a general review of *Aedes* egg hatching phenomena), an interaction that may have a profound influence on the likelihood of success of *Ae. albopictus* and *Ae. bahamensis* as colonists of tire habitats. Field studies of *Ae. albopictus* with *Ae. aegypti* in artificial habitats, tires in Texas (Sprenger and Wuithiranyagool 1986) and cemetery vases in Florida (O'Meara 1990) indicate initial success by *Ae. albopictus*, with a concomitant reduction of *Ae. aegypti* populations.

While *Ae. albopictus* has been spreading to the north and east from its apparent point of introduction in Texas, *Ae. bahamensis* populations of exotic origin, the Bahamas, have been detected in tires and other artificial containers in southern Florida (O'Meara et al. 1989), which renders the potential interactions among *Ae. albopictus*, *Ae. aegypti* and *Ae. bahamensis* in artificial containers all the more interesting. Although *Ae. bahamensis* has some potential as a disease vector, its eventual success in parts of North America may be beneficial if it can reduce the abundance of *Ae. aegypti*, a more competent vector (Llewellyn et al. 1970), or *Ae. albopictus*. Spielman and Feinsod (1979) reported an apparent exclusion of *Ae. aegypti* by *Ae. bahamensis* in the Bahamas, and field observations by O'Meara et al. (1989) in Florida also suggest that *Ae. aegypti* may be displaced in certain areas.

Problems of tire disposal have resulted in their accumulation on residential property and roadsides, in small rural and urban dumping sites, and in large commercial enterprises devoted to tire recycling or disposal. In forested areas, tires are inhabited by a fauna that resembles the community of treeholes located at the bases of trees that are likely to be found in the same forest. For example, a recent collection of tires in a Mississippi forest (Livdahl, unpubl.) yielded many *Ae. triseriatus*, as well as *Ae. albopictus*, *Orthopodomyia signifera* and predaceous *Toxorhynchites rutilus*, but collections from tires in sun-exposed areas in Mississippi yielded only *Ae. albopictus* and *Ae. aegypti* (personal observation). A full understanding of the *Ae. albopictus* invasion will require analyses of interactions within at least two types of communities, including treeholes and tires. The experiments presented here focus only on interactions in tire habitats, and use techniques developed for two prior studies.

The speed with which dominance was achieved suggests that interactions in addition to competitive displacement may have contributed to the establishment of *Ae. albopictus*. A recent experiment examining the responses of *Ae. triseriatus* and *Ae. albopictus* to density combinations (Livdahl and Willey 1991) suggests that competition with *Ae. triseriatus* will be insufficient to prevent the establishment of *Ae. albopictus* in treeholes, and that the two species have the potential for a stable coexistence in treehole communities; local extinction of *Ae. triseriatus* in tire habitats was predicted from these results, as well.

In this project, we examined competition between *Ae. albopictus* and *Ae. aegypti* in tires. Unlike the previous study, this experiment was performed in the field. Unlike previous studies of competition between *Ae. aegypti* and *Ae. albopictus* (lab study by Black et al., 1990; field study by Ho et al. 1989), the medium for this experiment was actual tire fluid with no artificial food or nutrient source added. Our criterion for determining the importance of larval competition in the displacement of *Ae. aegypti* was that the results should support a prediction of *Ae. aegypti*'s extinction, or at least a drastic reduction of *Ae. aegypti*'s equilibrium density. Other possible outcomes, such as stable coexistence or the exclusion of *Ae. albopictus*, would lead to the rejection of the competition hypothesis.

Interspecific egg hatch inhibition was examined by Edgerly, Willey and Livdahl (1993), who exposed single species *Aedes* egg batches to larvae of *Ae. aegypti*, *Ae. albopictus*, or *Ae. triseriatus* larvae at several densities in laboratory vials. For all species, the patterns of egg hatch were modulated by combinations of egg and larva species. Unlike eggs of *Ae. triseriatus* and *Ae. aegypti*, a relatively large fraction of *Ae. albopictus* eggs hatched despite high larval densities, regardless of larval species. Other hatch pattern differences resulted from differential effects of larvae; *Ae. albopictus* larvae suppressed hatching in interspecific interactions at levels greater than or equal to either of the resident species depending on density. Differential hatching inhibition by one species on the other could present an obstacle for colonization by *Ae. albopictus* or *Ae. bahamensis* or a mechanism for the eventual decline of *Ae. aegypti*.

We chose to test for this phenomenon in the field, in tire habitats, using *Ae. albopictus*, *Ae. aegypti* and *Ae. bahamensis*. Our criteria for determining the potential importance of this interaction to changes in the community of tire-breeding *Aedes* was that there must be asymmetries among these species in either the effects of larvae or the sensitivities of eggs, and that the interaction between *Ae. albopictus* and *Ae. aegypti* should favor *Ae. albopictus*. Results other than these would allow us to reject egg hatch inhibition as a major factor in the displacement of *Ae. aegypti* by *Ae. albopictus*.

To establish the relevance of these studies to natural populations, we have also conducted a field survey of urban and rural tire habitats within eight Florida counties with the objective of estimating the larval density of the *Aedes* species in areas that have been colonized by *Ae. albopictus* for different periods of time (0-7 years). We were particularly interested in testing for the possibility that *Ae. albopictus* is fluctuating about an equilibrium density in areas where it has been established for the longest periods.

iv. Materials and Methods

Sampling survey

County selection. Counties were selected on the basis of the period of time since *Ae. albopictus* was first discovered within each county. We sought a set of counties that would

provide the widest possible range of times since establishment, using information provide by G. O'Meara from his extensive statewide surveys. Counties selected for this survey, and the times since *Ae. albopictus* discovery, included Duval (7 years), Alachua (5 years), St. John's (4 years), Volusia and Indian River (3 years), Okeechobee (2 years), Martin (1 year) and Broward, which had no record of *Ae. albopictus* prior to this study. For all but one of the counties, samples were taken in early June, early August and late October, 1993. Okeechobee County was not sampled in June.

Site selection. We selected sampling sites without any advance knowledge of what sites would be most favorable to any of the three *Aedes* species. The basic method for selecting sites was to travel along Interstate 95, identify a highway exit near the latitudinal center of each selected county, travel to the vicinity of U.S. Route 1, which is heavily developed in many areas throughout Florida, to locate an urbanized source of tires for sampling. After locating an urbanized site, we travelled west along the nearest highway until signs of agricultural activity were discovered. Tires within such areas were considered rural habitats. Counties in which we could not follow this plan included Okeechobee County (no clearly urbanized areas, and neither U.S. Route 1 or Interstate 95 pass through Okeechobee County), Alachua County (no U.S. Route 1 or Interstate 95) and Volusia County, where no discarded tires seen in urban areas.

Sampling. It was normally possible to obtain ten tires for sampling at each site. Samples were either 1 l of tire fluid, unless the tire contained less than 1 l, in which case the entire fluid contents were taken. Fluid was removed by scooping water with a flexible plastic cup while agitating the tire vigorously. Samples were returned to the laboratory, where we waited for all the adults to emerge from each.

Analysis. The mean density of samples at each site was calculated after converting the number of adults emerged per unit volume from each sample with the $\ln(y+1)$ transformation. This rendered the data more nearly normal in their distribution, and allowed negative samples to be included in the analysis. Analysis of variance techniques were used to examine the influence of time since *Ae. albopictus* discovery, the influence of local development (urban or rural), and the interaction between these factors.

Competition Experiment

Experimental animals. Fresh laboratory populations of *Ae. albopictus* and *Ae. aegypti* were established from adults emerging from the sampling study. Eggs produced by these adults (F1 generation) were hatched in nutrient broth solutions to begin the experiment. The first two sets of replicates were hatched on July 17, 1993; larvae for additional replicates were hatched on July 28, 1993.

Experimental design. Initial densities of each species were fixed at 30, 45 and 60 individuals per 250 ml fluid. Larvae growing at all possible combinations of these densities were reared, with generally five replicates per density combination. Excess *Ae. aegypti* larvae permitted 8 replications of the 30/0 (*aegypti/albopictus*) and 7 of the 45/0 treatment combination; a shortage of *Ae. albopictus* larvae generally restricted the number of replicates including that species to four, although five replicates were obtained for the 45/45, 45/60, 60/30 and 60/45 treatment combinations.

Habitats. Used motorcycle tires were sawn into segments (6 per tire), and these segments housed the developing larval populations. Tire fluid was obtained from twenty tires in the Vero Beach area. It was pooled, larvae were removed from it, and the remaining fluid was stirred vigorously while 250 ml aliquots were disbursed into each tire segment. Newly-hatched larvae were then added, and these segments were monitored for pupae and adult emergence.

Tire segments were placed in an open shed, enclosed by chain-link fence. This arrangement provided natural temperature and light conditions, while preventing potential damage from vertebrates or heavy rains. Evaporative water loss from the segments was replaced on a weekly basis.

Rearing. Initially, the tire segments were individually enclosed by fiberglass screen, and emergent adults were removed every 2 d by aspirator. This proved unmanageable as the number of adults increased, and the screens were removed in early September. Thereafter, pupae were removed every 2 d and placed individually in emergence vials until all larvae had either died or emerged. The experiment ended on Oct. 29.

Analysis. The size of emergent females of each species was assessed by measuring wings to the nearest 0.03 mm with a dissecting microscope (35X magnification) equipped with an ocular reticle. Per capita growth rates were estimated for all larval cohorts from the composite index (Livdahl and Sugihara 1984)

$$r' = \frac{\ln \left[\frac{1}{N_0} \sum_x A_x f(w_x) \right]}{D + \frac{\sum_x x A_x f(w_x)}{\sum_x A_x f(w_x)}} \quad [1]$$

in which N_0 represents the initial number of females, A_x the number of females emerging on day x , and w_x the mean wing length of females emerging on day x ; $f(w_x)$ predicts number of female offspring based on female body size; and D estimates the delay between female emergence and first oviposition. Separate studies of individual females, offered blood daily after emergence, provide estimates of $D=9.5$ days for *Ae. aegypti* and $D=14.2$ days for *A. albopictus*. Linear functions describe size-dependent fecundity: $f(w_x)=31.2w_x-34$ for *Ae. aegypti* ($r^2=0.24$) and $f(w_x)=17.2w_x-14$ ($r^2=0.03$) for *Ae. albopictus*.

Regression of r' on the initial densities of *A. triseriatus* and *A. albopictus* can be expressed in the form

$$r'_i \approx \frac{dN_i}{N_i dt} = r_{mi} + b_i N_i + b_j N_j \quad [2]$$

When $(r_{mi} + b_i N_i + b_j N_j)$ is set equal to zero and N_j is plotted as a function of N_i , the result is a line that defines the combinations of initial densities that result in no population growth for species i , the zero growth isocline. Density combinations falling below an isocline permit continued growth of the species associated with that isocline; density combinations above an isocline will result in the species' decline. By comparing the positions of the two species isoclines, we determined the eventual outcome of the interaction if competition among larvae is the only interaction occurring between these species. If the isoclines intersect, the species can coexist in a stable manner. If one species' isocline falls completely outside the other, that species will drive the other extinct through larval competition.

Interspecific egg hatch inhibition

Experimental animals. Larvae and eggs of *Ae. aegypt*, *Ae. albopictus*, and *Ae. bahamensis* were the F1 generation of laboratory colonies initiated by field-collected larvae in June, 1993. Females oviposited on paper towels, which were cut into small irregular polygons with the objective of creating experimental egg batches with approximately 20 eggs per batch. Larvae were hatched and reared with abundant food (brewer's yeast) until they reached the fourth instar, at which time the experiment was initiated. All egg batches had been produced at least 14 d prior to the experiment.

Habitat . Motorcycle tires were circumcised about the tread to produce two open halves. Into each half, we inserted plastic walls with silicone caulk to create experimental compartments with 100 ml capacity. These compartments housed the tire fluid, the egg batches, which were pinned to the center of each compartment, and the larvae. The tires were placed on a roadside at the Florida Medical Entomology Laboratory in Vero Beach, Florida, and were covered with black plastic to minimize disturbance from rain.

Medium. Tire fluid was obtained from 20 tires in the Vero Beach area. Larvae were removed from the fluid, and the fluid was dispensed into the compartments while stirring vigorously.

Egg treatment. Eggs were submerged within experimental compartments for 24 h, after which they were removed, blotted dry, and examined to determine the number of eggs hatched and unhatched, and the number of fertile unhatched eggs.

Experimental design. Eggs of each species were exposed to larvae of each species at densities of 8, 24 and 48 per 100 ml. Control batches for each species were submerged in compartments with no larvae added. Six replicates of all treatment combinations were constructed, for a total of 180 egg batches and compartments. Analysis of variance was performed on hatch rate ($\text{asin}\sqrt{y}$ transformation) to examine the influence of egg species, larval species, and larval density.

v. Results

Sampling Survey

Density of Aedes aegypti. *Aedes aegypti* densities were significantly affected by the time since discovery of *Ae. albopictus*, and by the urban or rural character of the sampling site. Counties more recently invaded by *Ae. albopictus* showed higher densities of *Ae. aegypti* ($F_{5,242}=13.2$, $p<0.001$), and urban sites had higher mean densities of this species overall ($F_{1,242}=29.6$, $p<0.001$). A significant interaction between these factors indicates that the decline of *Ae. aegypti* has been more rapid in rural than in urban sites, as can be seen in Fig. 1. This analysis is based only on the June and August samples; the October samples are still being reared. It excludes data from Okeechobee county, which did not include an urban site and which was not sampled in June.

These data support the hypothesis that *Ae. albopictus* has excluded *Ae. aegypti* from the northern counties, where it has been established for the longest periods.

Density of Aedes albopictus. Density patterns for this species are nearly the mirror image of those for *Ae. aegypti* (fig. 1). Time since establishment ($F_{5,242}=4.8$, $p<0.001$) and the urban or rural nature of the site ($F_{1,242}=14.9$, $p<0.001$) were significant factors, as was the interaction between them ($F_{5,242}=3.4$, $p<0.01$). For this species, density was highest in the counties where it has been present the longest, and in rural sites. The southward advance of *Ae. albopictus* has been more rapid in rural sites than in urban sites, as shown by the significant interaction.

These data also support the hypothesis that *Ae. albopictus* is now fluctuating about an equilibrium density in the northern counties. No significant differences were found among the densities at rural sites in counties colonized during or before 1990. In urban sites, *Ae. albopictus* appears to have taken longer to reach an equilibrium density; no significant differences were found for counties colonized during or before 1988 (Duval and Alachua).

Density of Aedes bahamensis. Too few tires were positive for this species to warrant a detailed analysis. We did find, however, that *Ae. bahamensis* was the most common *Aedes* species in rural Broward County tires. They were found at no other sites in this survey.

Competition experiment

The following analysis provides clear evidence to reject the hypothesis that the displacement of *Ae. aegypti*, illustrated so clearly in Fig. 1, has not resulted from competition between the larvae of these two species. Each species showed a significant negative response to its own density, and neither species responded significantly to the density of the other species (table 1).

Table 1. Responses of per capita growth rate estimates for each species to the initial density of *Aedes aegypti* and *A. albopictus* larval cohorts in tire fluid. Regressions are based on Eq. 2. Standard errors for each regression term are shown. Only the conspecific regression coefficients (b_1) are significantly different from zero ($P < 10^{-3}$). Units are as follows: r_m , days⁻¹; b_1 and b_2 , days⁻¹•(individuals/250 ml)⁻¹. The numbers of r' values obtained for each regression (n) differ because some cohorts produced no survivors..

Species	n	r_{m_i}	b_1	b_2
<i>A. aegypti</i>	49	0.0299±0.0072	-0.0004±0.0002	-0.00005±0.00007
<i>A. albopictus</i>	50	0.0348±0.0069	-0.0005±0.0001	-0.00006±0.00008

These regressions permit the construction of zero growth isoclines for each species (fig. 2), which indicates that interspecific competition as a single factor is not important in the advance of *Aedes albopictus*: the two species should achieve a state of equilibrium coexistence at the intersection between the two isoclines, a result that clearly conflicts with the displacement pattern of densities shown in Fig. 1.

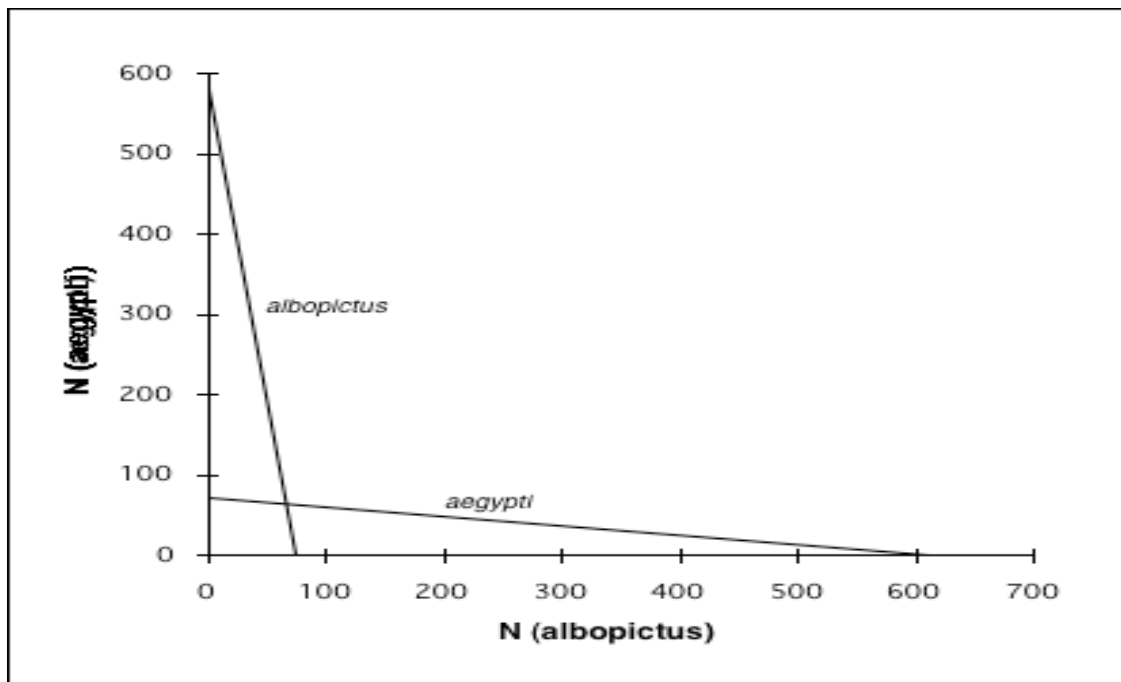


Fig. 2. Zero growth isoclines for *Ae. albopictus* and *Ae. aegypti*. Each line summarizes, for each species, the combinations of densities (in units of initial individuals per 250 ml) that permit zero population growth. Density combinations below each line constitute density conditions leading to growth; combinations outside the lines should result in population declines. The long-term outcome of this interaction should be coexistence at the point of intersection.

Interspecific egg-hatch inhibition

Species-specific larval influence and egg sensitivity. An initial analysis of hatch rates indicated that larval species was not a significant factor, either by itself ($F_{2,135} = 2.05$, $p > 0.13$) or in an interaction with density ($F_{4,135} = 0.40$, $p > 0.80$). That is, fourth instar larvae of *Ae. aegypti*, *Ae. albopictus* and *Ae. bahamensis* appear to have interchangeable effects on hatch rate. To permit a more thorough examination of egg sensitivity to larvae, the larval species factor was deleted; this allowed the incorporation of control egg batches into the analysis, which could not otherwise be assigned to a larval species group. The response of each egg species to larval density was then assessed with a two-way analysis of variance incorporating effects due to egg species, larval density, and an interaction between those factors. This analysis revealed a significant density effect ($F_{3,167} = 3.28$, $p < 0.05$) and a significant effect due to egg species ($F_{2,167} = 16.73$, $p < 0.001$), but no significant interaction between egg species and density

($F_{6,167}=0.54$, $p>0.77$), indicating that the sensitivities of these species' eggs to larval density are similar.

The egg species effect results from higher overall hatch rates for *Ae. bahamensis* relative to the other two species, which appear to have comparable overall hatch rates. The responses of all three species to larval density are roughly parallel, as shown in Fig. 3.

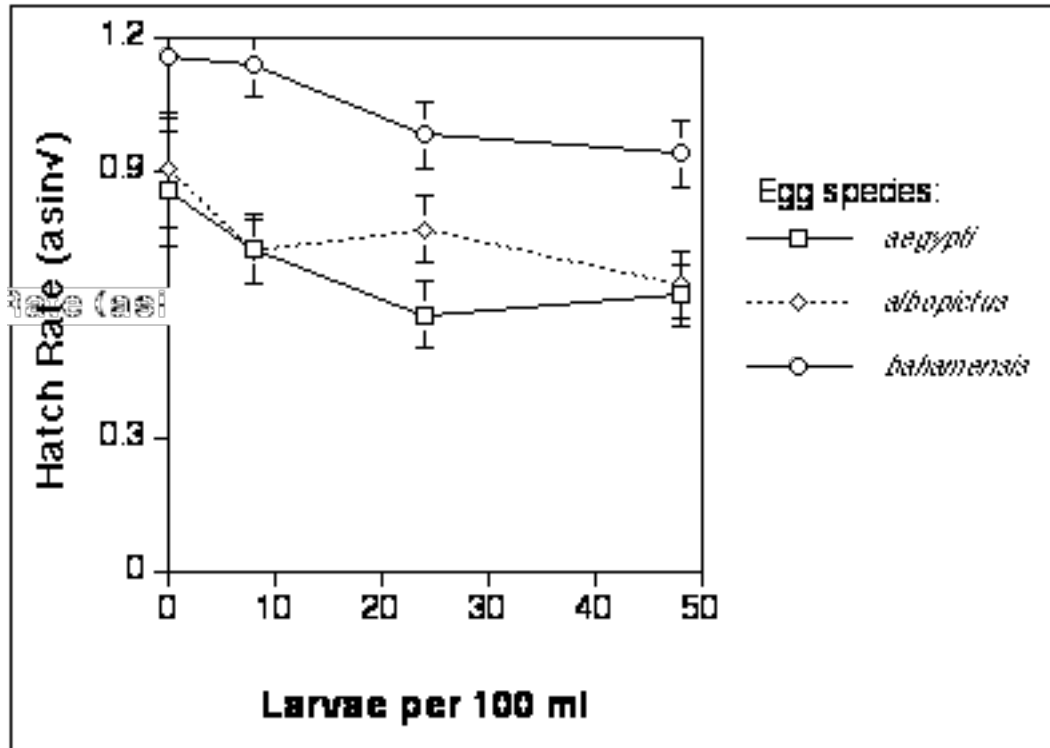


Fig. 3. Mean hatch rates (\pm SE) for three tire-breeding *Aedes* species as they respond to the density of fourth instar larvae in tire fluid. Means have been pooled across the three larval species. Hatch rates have been transformed by the arcsin square root method to homogenize variances and normalize distributions.

vi. Discussion

Our sampling results support the hypothesis that displacement of *Ae. aegypti* by *Ae. albopictus* is well underway. We have no reason to suggest, based on the sampling results, that this displacement will not continue to proceed southward throughout the peninsula of Florida. The more rapid displacement in rural sites is intriguing, but not explainable from the results of this project.

A more thorough sampling program would be in order at this point. The present study was limited by time and personnel, due to the demands of experimental phases of the project. In particular, the study would benefit from multiple urban and rural sampling sites within each county. At present, although the pattern appears clear, we cannot formally distinguish between site-to-site variation and county-to-county variation.

Broward County will be an interesting place to sample intensively during the next few years. We discovered *Ae. albopictus* larvae in our urban site in our October samples (not presented in the analysis), and an encounter between *Ae. albopictus* and *Ae. bahamensis* seems inevitable.

Based on the sampling data alone, any ecologist trained in theoretical community analysis would hypothesize that the displacement of *Ae. aegypti* has resulted from larval competition with *Ae. albopictus*. The larvae appear to have very similar resource requirements, and the adults appear to have very similar oviposition choices. However, the results from our competition study clearly point in other directions. We have no evidence that the larvae have any overlap in their resource requirements. Neither species had a significant impact on the other. This failure to detect an interspecific effect did not result from insufficient replication: responses of each species to its own density were clearly shown by this experiment. Although the competitive interaction still has not been completely explored (e.g. no studies have yet looked at the interspecific effects of large larvae on newly-hatch larvae), it appears that we will need to look elsewhere to account for the displacement. Possibilities include mating interference of *Ae. aegypti* by *Ae. albopictus* males, repulsion of ovipositing female *Ae. aegypti* by *Ae. albopictus* larvae, and differential susceptibility of these species to pathogens or predators. In addition, the

possibility remains that *Ae. aegypti* has declined in northern Florida due to factors entirely unrelated to the introduction of *Ae. albopictus*.

The list of alternatives to competition as explanatory factors should no longer include egg hatching inhibition by larvae. Our data show no strong differences in the response of *Ae. albopictus* and *Ae. aegypti* eggs to larval density, and no differences in larval inhibitory tendency. Although the inhibitory effect of larvae is supported by our results, it appears that this interaction may have importance only as a contributory factor that could amplify the effects of some other adverse effect that *Ae. albopictus* may have on *Ae. aegypti*.

vii. Literature Cited

- Black, W. C., K. S. Rai, B. J. Turco and D. C. Arroyo. 1989. Laboratory study of competition between United States strains of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 26: 260-271.
- Bradshaw, W.E. and C.M. Holzapfel. 1983. Predator mediated, nonequilibrium coexistence of tree-hole mosquitoes in southeastern North America. *Oecologia* 57: 239-256.
- Edgerly, J.S., Willey, M. and T. Livdahl. 1993. The community ecology of *Aedes* egg hatching: implications for a mosquito invasion. *Ecological Entomology* 18: 123-128.
- Gillett, J.D., E.A. Roman, and V. Phillips. 1977. Erratic hatching in *Aedes* eggs: a new interpretation. *Proc. R. Soc. Lond. B.* 196: 223-232.
- Grimstad, P.R. and E.D. Walker. 1991. *Aedes triseriatus* (Diptera: Culicidae) and LaCrosse Virus. IV. Nutritional deprivation of larvae affects adult barriers to infection and transmission. *J. Med. Entomol.* 28: 378-386.
- Hawley, W.A., P. Reiter, R.S. Copeland, C.B. Pumpini and G.B. Craig, Jr. 1987. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. *Science* 236: 1114 - 1116.
- Hawley, W.A., C.B. Pumpuni, R.H. Brady and G.B. Craig, Jr. 1989. Overwintering survival of *Aedes albopictus* (Diptera: Culicidae) eggs in Indiana. *J. Med. Entomol.* 26: 122-129.
- Livdahl, T. and J.S. Edgerly. 1987. Egg hatching inhibition: field evidence for population in a treehole mosquito. *Ecol. Entomol.* 12: 395-399.
- Livdahl, T., R. Koenekoop and S. Futterweit. 1984. The complex hatching response of *Aedes* eggs to larval density. *Ecol. Entomol.* 9: 437-442.
- Livdahl, T. and R. Koenekoop. 1985. The nature of egg hatching in *Aedes triseriatus* : ecological implications and evolutionary consequences. In *Ecology of Mosquitoes*:

- proceedings of a workshop* (L.P. Lounibos, J.R. Rey and H. Frank, Eds.), pp. 439-458.
Florida Medical Entomology Laboratory, Vero Beach, FL.
- Livdahl, T. and M. Willey. 1991. Prospects for an invasion: competition between *Aedes albopictus* and native *Aedes triseriatus*. *Science* 253: 189-191.
- Llewellyn, C.H., A. Spielman and T.E. Frothingham. 1970. Survival of arboviruses in *Aedes albopictus*, a peridomestic Bahaman mosquito. *Proc. Soc. Exp. Biol. Med.* 133: 551-554.
- Nawrocki, S. J. and W. A. Hawley. 1987. Estimation of the northern limits of distribution of *Aedes albopictus* in North America. *J. Am. Mosq. Con. Assoc.*3: 314-317.
- O'Meara, G. 1990. The spread of *Aedes albopictus* and *Aedes bahamensis* in Florida. Paper, Entomological Society of America annual meeting, New Orleans, LA.
- O'Meara, G. F., V. L. Larson, D. H. Mook and M. D. Latham. 1989. *Aedes bahamensis*: its invasion of south Florida and association with *Aedes aegypti*. *J. Am. Mosq. Con. Assoc.*5: 1-5.
- Pumpuni, C.B., Hawley, W.A. and G.B. Craig. 1989. Critical photoperiod in *Aedes albopictus*: effect of temperature and latitudinal origin. Paper presented at the 1989 Annual Meeting of the Entomological Society of America.
- Spielman, A. and F.M. Feinsod. 1979. Differential distribution of peridomestic *Aedes* mosquitoes on Grand Bahama Island. *Trans. R. Soc. Trop. Med. Hyg.* 73: 381-384.
- Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J. Am. Mosq. Con. Assoc.* 2: 217-219.

viii. Acknowledgements

We thank the Florida Department of Agriculture and Consumer Services, the Bureau of Entomology and Pest Control and the Department of Environmental Regulation, Solid Waste Management Trust Fund for supporting this project. The Department of Biology at Clark University provided funds for supplies. We also thank Lester Scherer of the Martin County Public Works Department, for providing mosquito control agency endorsement of the project proposal. We are especially grateful to Dr. James Baker, Director of the Florida Medical Entomology Laboratory, for providing generous access to facilities and lodging at the laboratory, and Dr. George O'Meara for valuable advice, assistance, and hospitality during our visits to Vero Beach.