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Oviposition habitat selection by container-dwelling mosquitoes: responses to cues of larval and detritus abundances in the field

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Abstract

1. Insects' oviposition responses to resource and larval densities can be important factors determining distributions and competitive interactions of larvae. *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.) (Diptera: Culicidae) show aggregated distributions of larvae in the field, larval interactions that are affected by detritus resources, and oviposition responses to resource and density cues in the laboratory. We conducted field experiments testing whether these species choose oviposition sites in response to chemical cues indicating detritus resource quantity and quality or larval abundances.
2. In experiment 1, both species showed interactive responses to water conditioned with high or low quantities of senescent live oak leaves and density combinations of *A. albopictus* and *A. aegypti* larvae. *Aedes aegypti* preferred high-detritus containers when conspecifics were absent. *Aedes albopictus* tended to prefer high-detritus containers when larval density was low. We found no evidence of interspecific differences in oviposition preferences.
3. In experiment 2, *A. albopictus* preferred high detritus over low or no detritus, and rapidly-decaying, high-quality detritus over low-quality detritus.
4. Oviposition choices by these *Aedes* are mainly determined by resource quantity and quality, with larval densities having minor, variable effects. Oviposition responses of these species are unlikely to lead to resource partitioning. Aggregated distributions of these species in the field are unlikely to be products of oviposition choices based on larval densities.

Keywords

oviposition choice; resource abundance; larval density; *Aedes aegypti*; *Aedes albopictus*; aggregation; resource partitioning

Introduction

Habitat selection theory predicts that organisms will disperse and colonize habitats in a way that maximizes expected fitness (Resetarits 1996, Binckley & Resetarits 2005, 2008, Resetarits & Binckley 2009). For species that provide no parental care to offspring deposited in discrete patches, selection on the ability to discriminate favorable and unfavorable patches

Contributions of authors

JEF and SAJ designed the experiments, analyzed the data, and wrote the manuscript. JEF conducted the field experiments and data collection.

should be particularly strong because offspring are confined to a patch until some developmental milestone is reached (Resatarits & Wilbur 1989). In many patchy-systems such as *Drosophila*-colonized fruits and mushrooms (e.g., Atkinson 1985) and aquatic container communities (e.g., Juliano 1998), resource competition within a patch is important and may be a significant source of larval mortality, hence female oviposition decisions should minimize the future experience of competition by her larvae. Container-dwelling mosquitoes seem particularly capable of responding to volatile chemical cues that may reflect current or future intensity of larval competition. For example, the presence of con- and heterospecific competitors (Zahiri et al. 1997, Edgerly et al. 1999) or the quantity and type of resources present in a container (Ponnusamy et al. 2008, Reiskind et al. 2009) can influence oviposition choices in these systems.

Aedes aegypti and *Aedes albopictus* are container-dwelling mosquitoes that inhabit human-dominated landscapes throughout the world. Both are competent vectors of multiple arboviruses and thus their reproductive and oviposition behaviours have received much attention (e.g., Sucharit et al. 1980, Allan & Kline 1998, Sharma et al. 2008). *Aedes aegypti* responds to cues from eggs and larvae of both conspecifics and heterospecifics, including *A. albopictus* (Zahiri et al. 1997, Allan & Kline 1998). Behavioural assays often test the response of gravid females to water conditioned by larvae or eggs although specific chemical cues have also been isolated and assayed in oviposition trials (Zahiri et al. 1997, Ganesan et al. 2006, Seenivasagan et al. 2009). *Aedes aegypti* females respond positively to chemical cues derived from well-fed and healthy larvae, but may be repelled by chemical cues from larvae at very high densities or from starved larvae (Zahiri et al. 1997, Allan & Kline 1998, Zahiri & Rau 1998, Seenivasagan et al. 2009). Cues indicating resource availability in a container also elicit oviposition responses from these species. Chemical cues associated with microorganisms from decaying organic matter elicit positive oviposition responses from both *A. albopictus* (Trexler et al. 2003) and *A. aegypti* (Benzon & Apperson 1988, Ponnusamy et al. 2008). Reiskind et al. (2009) reported oviposition preference of *A. albopictus* for containers with oak leaves over containers with grape leaves or plain water. In larval competition experiments oak leaves are a superior resource compared to grape leaves, suggesting that *A. albopictus* selects oviposition sites that will reduce larval competition and maximize expected fitness (Reiskind et al. 2009).

These observations suggest that ovipositing container-dwelling *Aedes* accurately respond to container conditions, including larval density and resources, as predicted by habitat selection theory. However, most studies have tested oviposition behaviour in the laboratory, under carefully controlled conditions. For example, oviposition assays have been conducted on multi-well plates with water volumes of only a few ml, and water treatments separated by several centimeters or less (Zahiri et al. 1997). Others have used electroantennogram techniques and Y-maze olfactometers to assess the mechanisms eliciting attraction to cues (i.e., behavioural orientation vs. stimulation to oviposit; Seenivasagan et al. 2009). These are effective methods for understanding the proximate causes of these behaviours and the range and strength of responses that can be expected in these species. These studies, as well as a select few field studies (e.g., Trexler et al. 1998), have been largely motivated by the goal of developing attractants or repellents for mosquito control and monitoring, important goals given the human health importance of these species. However, relatively little is known about the importance of such chemical cues in the presence of natural variability, and how they might influence distributions of mosquito eggs across oviposition sites and resulting ecological interactions among larvae. The strong and species-specific responses observed in laboratory experiments may be important drivers of species distributions in wild populations, particularly as they affect densities of individuals relative to local resource availability, and spatial variation of competitive interactions. Understanding responses to

these cues in the field and their potential for affecting actual distributions of species, is the goal of this paper.

Aedes albopictus and *A. aegypti* coexist in some areas of the southern United States (O'Meara et al. 1995) despite competitive asymmetry usually favoring *A. albopictus* (Juliano 1998, 2009, 2010). A leading hypothesis to account for this pattern is condition-specific competition which suggests that the outcome of competition depends on the conditions within larval containers (Juliano 2009, 2010). For example the abundance of rapidly decaying detritus releases *A. aegypti* from interspecific resource competition with *A. albopictus* (Daugherty et al. 2000, Murrell & Juliano 2008, Juliano 2010, O'Neal & Juliano 2013). Based on the oviposition experiments discussed above, one hypothesis for coexistence of these species postulates that *A. aegypti* females select container habitats with higher levels of high-quality resources, stabilizing coexistence via resource partitioning (Shorrocks & Sevenster 1995, Adler et al. 2007). This hypothesis predicts that the two species will show different oviposition responses to detritus resource quantity and quality in the field, and negative correlations of egg numbers. Another coexistence hypothesis in this system is the 'aggregation model' of coexistence (Atkinson 1985, Ives 1991) that postulates that intraspecifically aggregated or clumped distributions of larvae among containers will increase intraspecific competition relative to interspecific competition, facilitating coexistence (Ives 1991, Shorrocks & Sevenster 1995, Fader & Juliano 2013). This hypothesis predicts that one or both species preferentially oviposit in containers containing conspecifics in the field. Both *A. aegypti* and *A. albopictus* preferentially oviposit in laboratory containers with conspecific larvae or eggs (Allan & Kline 1998) and if this behaviour is prevalent in nature this would directly increase intraspecific aggregation in the field and the likelihood of coexistence (Ives 1991). Aggregated spatial distributions of these species have been observed in the field suggesting aggregation may affect interspecific competition and coexistence (Leisnham & Juliano 2009, Fader & Juliano 2013). These predictions center on oviposition behaviour in response to ecologically realistic cues from detritus or conspecifics at relevant spatial scales in the field. In this paper we describe two experiments testing these hypotheses about oviposition responses of naturally-occurring *A. albopictus* and *A. aegypti* females to larval abundance and detritus resource quantity and quality cues at levels likely to be encountered in nature.

Methods

For both experiments resource quantities and larval densities were determined based on observations of actual vases in Florida cemeteries (Fader & Juliano 2013).

Experiment 1: Larval and detritus cues

To test for effects on oviposition of cues from developing larvae of *A. albopictus* and *A. aegypti* at two levels of resource availability, larval rearing water (LRW) was prepared by allowing larvae at various intraspecific and interspecific densities to develop within plastic containers. Larvae and detritus were removed from the containers and remaining LRW used in oviposition assays in the field. Experiment 1 was a two-way factorial design with two levels (high or low) of resource (live oak leaves, *Quercus virginiana* Mill.), and seven density combinations of *A. albopictus*-*A. aegypti* larvae (0-0, 10-10, 20-0, 0-20, 20-20, 40-0, and 0-40 larvae per container).

Experimental containers were 400 ml plastic cups with 250 ml distilled water. Each container received 0.25 g or 0.50 g (± 0.003 g) of senescent *Q. virginiana* leaves collected in 2009 from Vero Beach, FL. Leaves were dried at 60°C for 48 h before addition to containers. *Aedes albopictus* and *A. aegypti* eggs obtained from laboratory colonies of field collected mosquitoes from Tampa, FL (F₁ generation) were synchronously hatched in a 0.15

g/l mixture of yeast/lactalbumin (1:1) and added to experimental containers in the above combinations three days after the containers had been established. Containers were housed in a screened building in a subtropical coastal oak hammock on the campus of the Florida Medical Entomology Laboratory (FMEL) in Vero Beach, FL.

Ten days after addition of larvae the entire contents of each container were passed through a 106 μm sieve and resulting LRW transferred to a plastic water bottle for transport to field locations. Oviposition assays of LRW were then conducted at three cemeteries within the city of Tampa, FL (Rose Hill Cemetery, North 25th street Cemetery, and Woodlawn Cemetery) in a randomized complete block design. All three cemeteries were expected to have populations of both *Aedes* species. Three complete blocks, consisting of 14 plastic green cemetery vases each (American Cemetery Supplies, Inc., Portsmouth, VA), were placed randomly in each cemetery. Each vase was lined with seed germination paper (Anchor Paper Co., St. Paul, MN) held in place with a clip as an oviposition surface for mosquitoes, and all 14 LRW treatments were randomly assigned to vases within a block. Treatments were placed in the field on 23 June 2009. On 30 June 2009 LRW and seed germination papers were collected. Germination papers were incubated for 4 days at 14 L: 10 D (fluorescent lighting), 23–26 °C in a climate-controlled building, and then hatched in 0.15 g/l mixture of yeast/lactalbumin. Hatched larvae were fed *ad libitum* with yeast/lactalbumin until identification was possible (3–6 days), and then numbers of each species determined. As *A. albopictus* and *A. aegypti* do not differ in egg survivorship, and eggs have very high survivorship (>88%, usually over 96% over a two-week period), under these incubation conditions (O’Neal & Juliano 2013) our hatches are a good indicator of the oviposition choices made by females. Mortality of larvae before identification was very low, and we are confident that our response variables reflect actual oviposition patterns and not interspecific differences in post-collection survival.

Experiment 2: Response to detritus type and amount

To separate the effects of resource-derived cues from larval cues on oviposition responses, a follow-up experiment in summer 2010 tested the response of *Aedes* mosquitoes to resource derived cues alone. The follow-up experiment tested responses to four different detritus types, all common in artificial containers and known to produce different competitive outcomes between *A. aegypti* and *A. albopictus* (Murrell & Juliano 2008). No larval cues were involved in this experiment. Three plant- and one animal-based detritus types were used: senescent live oak leaves (*Q. virginiana* Mill.); senescent slash pine needles (*Pinus elliotti* Engelm.); fresh grass clippings (*Zoysia* sp.); and freeze-killed crickets (*Grylodes sigillatus* (Walker)). Crickets were obtained from laboratory colonies at Illinois State University and all plant resources were obtained on the grounds of FMEL in Vero Beach, FL in 2010. All detritus types were dried at 60°C for 48 hours and then added to 450 ml distilled water in 500 ml NALGENE® bottles. Treatments consisted of high (plant detritus: 1.0 ± 0.002 g; insect detritus: 0.2 ± 0.002 g) and low (plant detritus: 0.2 ± 0.002 g; insect detritus: 0.04 ± 0.002 g) detritus quantities. A lower amount of insect detritus was used because large quantities of animal material foul containers very rapidly (Murrell & Juliano 2008). A distilled water control was treated identically except for addition of detritus, for a total of nine unique treatments (four resources, high and low quantities, plus 1 distilled water control).

Oviposition assays for detritus cues began two days following establishment of containers. Water treatments including detritus were placed in plastic cemetery vases lined with germination paper and arranged in a randomized complete block design as described in Experiment 1. Eight blocks were placed in Woodlawn Cemetery in Tampa, FL on 2 July, 2010 and collected on 8 July, 2010. Eggs were incubated as in experiment 1 for eight days

and then hatched in a 0.15 g/l solution of yeast/lactalbumin. Larvae were reared on *ad libitum* yeast/lactalbumin and quantified as in experiment 1.

Statistical Analysis

Generalized Linear Mixed Model ANOVA (PROC GLIMMIX, SAS Institute 2008) with a randomized complete block design was used to analyze responses of ovipositing *A. albopictus* and *A. aegypti* to water treatments. Blocks were modeled as random effects in all experiments, and were nested within cemetery (also a random effect) in experiment 1. Significant effects were further analyzed by contrasts of least squares means at Bonferroni experimentwise $\alpha=0.05$. The number of larvae of each species hatched from a treatment container served as the dependent variable for all three experiments, and was analyzed using a Poisson distribution of error and a log link function. In experiment 1 only, differential responses of the two species to density and resources were tested by analyzing the difference in number of eggs (*A. albopictus* – *A. aegypti*) using a Normal distribution of error and identity link function. Significant effects in this analysis would indicate that density combination, resource amount, or interaction differentially affect oviposition by these species (e.g., if one species prefers high resources and the other low resources, the difference will be large for one resource treatment and small for the other). Also for experiment 1 only, Pearson correlations of numbers of eggs of the two species within each of the three cemeteries were tested. Experiment 1 was analyzed with resource (high and low), larval density treatments, and interaction as fixed effects. The design was unbalanced due to loss of a single vase during the assay. In experiment 2 there was virtually no oviposition by *A. aegypti* (see **Results**); hence only responses of *A. albopictus* could be analyzed for this experiment. Experiment 2 was analyzed with treatment (detritus type or larval density, respectively) as a fixed effect.

Results

Experiment 1

Numbers of eggs laid by *A. albopictus* and *A. aegypti* were significantly affected by resource quantity, larval density combination, and the interaction (Table 1). Because of the significant interaction, we focused on comparing means in two ways: first we compared mean numbers of eggs laid at high and low resources quantities, for the same larval densities; second we compared all possible pairs of larval densities within each resource quantity. For *A. albopictus*, response to resource quantity was inconsistent. For 0-0 and 10-10 *A. albopictus*-*A. aegypti* combinations, *A. albopictus* laid significantly more eggs in high-resource containers, but for the 0-20 *A. albopictus*-*A. aegypti* combination, *A. albopictus* laid significantly more eggs in low-resource containers (Fig. 1A). For all other density combinations there was no significant difference due to resource amount (Fig. 1A). Among the low-resource treatments, the 0-20 combination received the most eggs, significantly more than all other combinations except 0-40 (Fig. 1A). The 0-40 combination received significantly more eggs than did 0-0 and 10-10 (Fig. 1A). Among high-resource treatments the 0-0 combination received the most eggs, significantly more than the 0-20, 0-40, and 40-0 combinations (Fig. 1A). For *A. aegypti*, response to resource quantity was more consistent and easily summarized. There were significantly more eggs laid in high-resource treatments that contained no *A. aegypti* (0-0, 20-0, and 40-0 *A. albopictus*-*A. aegypti* combinations; Fig. 1B). Within the low-resource treatments, 10-10, 20-20, and 40-0 combinations received the most eggs, significantly more than the 0-0 combination (Fig. 1B). Within the high-resource treatments, the 40-0 combination received the most eggs, followed by the 20-0, 20-20, and 0-0 combinations, and all four of these combinations yielded significantly more eggs than did the 0-20 combination (Fig. 1B). Also at high-resource levels, the 40-0 combination yielded significantly more eggs than did the 0-40 and 10-10 combinations (Fig. 1B).

Analysis of the difference in oviposition between the two species yielded no significant effects of detritus resource ($F_{1,103}=0.53$, $P=0.4683$), density combination ($F_{6,103}=1.74$, $P=0.1198$), or interaction ($F_{6,103}=0.92$, $P=0.4835$), indicating that these treatments did not affect species oviposition choices differentially. Oviposition of the two species was significantly positively correlated in one of three cemeteries: North 25th street $r=0.379$, $N=41$, $P=0.0146$; Woodlawn $r=0.217$, $N=42$, $P=0.1768$; Rose Hill $r=-0.066$, $N=42$, $P=0.6776$.

Experiment 2

Aedes aegypti females were apparently absent at Woodlawn Cemetery in summer 2010 and thus analyses for experiments 2 and 3 include only the oviposition response of *A. albopictus*. Resource treatments significantly affected *A. albopictus* oviposition (Table 2). For further analysis of this experiment, we used contrasts to evaluate effects of resource amounts (None, High, Low), resource types (Coniferous, Deciduous, Grass, Insect), and the interaction of type with amount (including only high and low). High resources and no-resource controls differed significantly (Table 2) with more eggs per container in the pooled high-resource containers (Fig. 2). Low resources and no-resource controls did not differ significantly (Table 2, Fig. 2). The effect of detritus type was also significant (Table 2), but the interaction of resource type and amount was not significant (Table 2). In the absence of this interaction, comparison of the main effects of resource amount (High vs. Low) and resource type is justified. High and low resource treatments pooled across types differed significantly (Table 2). High resources attracted more oviposition than did low resources, and this was consistently true for all resource types (Fig. 2). For resource types, oviposition was significantly greater for insects and grass than for coniferous and deciduous (Table 2, Fig. 2). Insect and Grass did not differ significantly, and Coniferous and Deciduous did not differ significantly (Table 2, Fig. 2). Deciduous leaves, the detritus type used in experiment 1, was the least attractive of all resource treatments (Fig. 2).

Discussion

Aedes albopictus and *A. aegypti* females are known to oviposit selectively in response to factors potentially reflecting container conditions for developing larvae. In particular, cues derived from decaying resources or from conspecific larvae have received attention as potential attractants for ovipositing mosquitoes. Such behaviours could also have important implications for intra- and interspecific larval competition and distribution patterns; however, many studies are designed with the goal of improving surveillance and monitoring, making it difficult to infer the importance of such behaviours for ecological interactions. Our experiments investigating whether wild, ovipositing mosquitoes in south Florida choose oviposition sites based on these same cues in nature, at concentrations similar to those in container habitats, suggest that the response to resource availability is likely to be more important than the response to larval density. However, our data were not consistent with the predictions of the resource partitioning and aggregation hypotheses. The two species did not show distinct responses to resource abundances in experiment 1, and neither species showed a strong response to conspecific density in experiment 1.

Though the numbers of eggs of each species deposited in our containers over one week may seem low (Figs. 1 and 2), they are consistent, and perhaps even slightly greater, than those observed in previous oviposition trapping studies conducted in Tampa, Florida cemeteries (Leishnam & Juliano 2009). In general, the responses of ovipositing females to cues of resource or larval presence in the field were less dramatic than in most laboratory studies. Our field experiments 1 and 2 yielded significant effects with both showing important effects of detritus resource quantity or type on oviposition. The quantities and types of

detritus used to generate cues in these experiments were based on field observations (Fader & Juliano 2013) indicating the potential importance of such cues in nature. Experiment 1 suggested that when it showed an oviposition preference, *A. aegypti* preferred oviposition in high-resource containers. The response of *A. albopictus* in experiment 1 often favored high-resource containers, but there was at least one prominent exception (Fig. 1A). The response of *A. albopictus* to detritus resource quantity was clearer in experiment 2, where they clearly preferred high resource levels, and preferred those detritus substrates (insect, grass) expected to yield greatest larval growth rate and bacterial abundance (Murrell & Juliano 2008, Trexler et al. 2003, Ponnusamy et al. 2008, Yee et al. 2006). These results, particularly the preference shown for higher quality or quantity of resources, are broadly consistent with the very strong and specific responses observed in previous studies (e.g., Reiskind et al. 2009). Because the two species show similar preferences for high detritus resources, oviposition responses seem likely to concentrate eggs in the most productive containers. Such similar responses by competitors would be unlikely to lead to resource partitioning and stabilizing effects on these competitors, but may contribute to coexisting by equalizing fitness (Adler et al. 2007).

Responses to larval densities were much more limited and only significant in experiment 1 as interaction effects. Even when we found significant effects of larval density combination in experiment 1, there was no clear pattern of preference for either high or low density of one or both species, with responses by both *A. albopictus* and *A. aegypti* to density apparently idiosyncratic. Responses to larval abundances may be mediated by larval effects on bacteria and thus dependent on detritus resource amounts, as in experiment 1. Past observations have shown responses to larva-derived cues (Zahiri & Rau 1998, Seenivasagan et al. 2008, Allan & Kline 1998); we regard our results concerning larval density as compatible with these earlier results, because we tested the simultaneous effects of resources and densities in the complex environment of the field). Our results raise questions about whether these sorts of cues are important determinants of the distributions of these *Aedes* in the field, at least under the conditions we observe in urban Florida. The absence of a consistent response to density in experiment 1 may be attributable to the limited time larvae were resident in containers (only 10 days). For ovipositing females, containers with a long history of successful growth and development of larvae (i.e., weeks or months), and associated build-up of larval-derived cues, may provide the most attractive oviposition substrates. In addition to tests of effects of larval density on oviposition attractants, it would be useful to test for effects of the time over which successful cohorts of larvae have developed in a container.

Our use of a randomized block design could be another factor contributing to the lack of strong, density-dependent patterns of oviposition. It is possible that volatile cues attracted females to the cluster of containers within a block, but females then simply spread eggs over multiple containers in that block regardless of within-container density, obscuring evidence for that attraction. Other aquatic insects show this type of oviposition response to clusters of attractive or unattractive oviposition sites (Resetarits & Binckley 2009). Laboratory studies of *Aedes* using much denser arrays of potential oviposition sites (e.g., well-plates) and finding selective oviposition argue against this possibility, however (Zahiri et al. 1997). More likely, variability in the field environment, including sun exposure, temperature, and proximity of resting, sugar-feeding, and blood-feeding sites, may also influence oviposition choices and could obscure all but the strongest oviposition responses to larval density. Our results are also tied to the environment in which we work (urban, subtropical cemeteries) and it would be interesting to investigate these kinds of oviposition responses in other environments where density of ovipositing females, or densities of containers, may be very different.

Our primary motivation for this study was to determine if selective oviposition behaviours are important in nature and potentially influence distributions, abundances, and competitive interactions between *A. albopictus* and *A. aegypti* in urban container communities. These species overlap in urban areas of south Florida despite competitive superiority of *A. albopictus* in controlled experiments (Juliano 1998, 2009, 2010), and differential habitat selection could make important contributions to coexistence. For example, detritus type influences competitive interactions between *A. albopictus* and *A. aegypti* with competitive release of *A. aegypti* in the presence of fast-decaying, high quality detritus sources such as insects or grass (Daugherty et al. 2000, Murrell & Juliano 2008, Juliano 2010). Our experiments provide some evidence for selective oviposition in nature by both species, and attractiveness of fast-decaying, high quality detritus resources to *A. albopictus*. If selective oviposition with favorable detritus types, similar to those shown by Reiskind et al. (2009) in *A. albopictus* and *A. triseriatus*, results in reduced impact of competition, then this response may contribute to species coexistence via fitness equalizing effects (Adler et al. 2007, O'Neal and Juliano 2013).

Aggregated or clumped distributions of larvae among containers have been observed for these two species (Leisnham & Juliano 2009, Fader & Juliano 2013), and may contribute to coexistence via increased intraspecific competition relative to interspecific competition (Fader & Juliano 2013, Ives 1991). This aggregation hypothesis differs from traditional resource partitioning by accounting for coexistence across similar patches (i.e., containers), but could also be greatly affected by habitat selection behaviours (Ives 1991). Indeed, laboratory evidence that larval and egg cues attract oviposition by female *Aedes* (e.g., Allan & Kline 1998) would appear to provide a very direct mechanism that could produce strong patterns of larval aggregation. Our results suggest, however, that preferential oviposition in response to larval density may not offer a robust explanation for aggregation patterns observed in nature. Other mechanisms such as clutch-laying by females, responses to egg density, aggregation in high resource patches, or differential mortality in containers should be explored. Of the two cues we investigated, resources, rather than conspecific or heterospecific densities, seem considerably more likely to affect oviposition, aggregation, and competition.

We observed oviposition responses to resource cues, but not to density cues, in the field, at cue concentrations that are realistic and at the spatial scale of the typical habitat of these species in south Florida. We suggest that oviposition preference behaviours related to detritus resource amount and quality are more important in determining local distributions and ecological interactions in urban environments, resulting in distributions of larvae that to some degree track food availability for larvae. As we found little evidence for a direct effect of larval density on oviposition choices, we suggest that aggregated larval distributions of these species in nature (e.g., Fader & Juliano 2013) are not likely the result of larval density cues, but rather, reflect differences in the capacity of different containers to support larvae and to produce adults, as suggested by Fader & Juliano (2013).

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Fig. 1.

Back transformed Least Squares means ($\pm$ SE) of number of (A) *A. albopictus* and (B) *A. aegypti* individuals sampled in cemetery vases at high (0.50 g) or low (0.25 g) live oak detritus treatments across three cemeteries in Tampa, FL (experiment 1). Means within a density combination marked with * differ significantly. Means within a detritus level marked with the same letters of the same case are not significantly different.

Fig. 2. Back transformed means ($\pm$ SE) of number of *A. albopictus* individuals sampled at Woodlawn Cemetery in Tampa, FL in cemetery vases with high (H, open bars) or low (L, grey bars) levels of one of four detritus types or a no-detritus control. For statistical tests of contrasts involving combinations of means, see Table 2.

Table 1

Mixed model ANOVA on oviposition responses of *Aedes* mosquitoes to larval density and resource-availability cues in experiment 1. Random effects included in the model: Cemetery; Block(Cemetery).

Source	<i>A. albopictus</i>			<i>A. aegypti</i>		
	df	F	P > F	F	P > F	
Resource	1, 103	7.23	0.0084	32.64	0.0001	
Density	6, 103	4.02	0.0012	11.54	0.0001	
Resource×Density	6, 103	11.22	0.0001	4.78	0.0002	

Table 2

Mixed model ANOVA on oviposition responses of *Aedes albopictus* to detritus resource type and amount in experiment 2. Contrasts shown below overall treatment effect. Contrasts shown in **bold face type** are statistically significant at experimentwise $\alpha=0.05$ (Bonferroni adjustment). Random effects included in the model: Block.

Effect	DF	F	Pr > F
Treatment	8	17.99	0.0001
None vs. High	1	11.91	0.0011
None vs. Low	1	0.08	0.7741
High vs. Low	1	37.47	0.0001
Detritus type	3	18.67	0.0001
Coniferous vs. Deciduous	1	6.71	0.0122
Coniferous vs. Grass	1	19.71	0.0001
Coniferous vs. Insect	1	10.30	0.0022
Deciduous vs. Grass	1	45.56	0.0001
Deciduous vs. Insect	1	31.92	0.0001
Grass vs. Insect	1	1.87	0.1766
Detritus type x amount interaction	3	2.42	0.0753
Error	56		