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## Behavioral Responses of *Aedes albopictus* to a Predator Are Correlated with Size-Dependent Risk of Predation

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### Abstract

The invasive container-dwelling mosquito *Aedes albopictus* (Skuse) shows modest behavioral responses to water-borne cues from predatory *Corethrella appendiculata* Grabham in North America. We investigate whether *Ae. albopictus* adjust their antipredatory responses to be proportional to size-dependent risk of predation. Fourth-instar *Ae. albopictus* attain a size refuge from *C. appendiculata* predation, and we compared the responses of second- and fourth-instar *Ae. albopictus* to cues from *C. appendiculata* predation. More vulnerable second-instar larvae showed a larger change in behavior in response to predation cues than did less vulnerable fourth-instar larvae, indicating threat-sensitive behavioral responses by *Ae. albopictus*.

### Keywords

*Aedes albopictus*; *Corethrella appendiculata*; predator-prey; size-dependent

In aquatic systems, prey often show behavioral modifications in response to predation risk, and these modifications reduce an individual's risk from predation. These responses to cues to predation risk typically come at a cost that can result in significant nonlethal predator impacts on the fitness of prey (Preisser et al. 2005). Because of these costs, we hypothesize that there should be behavioral responses that are proportional to the actual risk of predation. Insufficient responses to highly dangerous predators would be selected against by increased mortality, and excessive responses to minimally dangerous predators would be selected against by costs of reduced foraging, movement, and growth. Because relative size of predators and prey can influence risk (Chivers et al. 2001), we expect prey individuals of different sizes to show different responses to the same predators.

Second instars of both the invasive *Aedes albopictus* and the native *Aedes triseriatus* (Say) mosquitoes reduce their activity at the bottom of containers in the presence of water-borne predation risk cues from *Corethrella appendiculata* Grabham, and these changes reduce the risk of predation (Kesavaraju et al. 2007). Second-instar *Ae. albopictus* show these behavioral modifications only in water that has held a feeding *C. appendiculata* and not to a nonfeeding predator (our unpublished data). The activity of *Ae. albopictus* changes less than that of *Ae. triseriatus*, so *Ae. albopictus* larvae are more vulnerable to predation from *Corethrella* (Kesavaraju et al. 2007). Second-instar mosquitoes are more vulnerable than are larger instars to predation by fourth-instar *C. appendiculata* (Kesavaraju et al. 2007). Thus, second-instar *Ae. albopictus* should show a greater change in behavioral responses to cues from predation by *C. appendiculata* than should the relatively invulnerable fourth-instar *Ae. albopictus*.

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## Materials and Methods

*Ae. albopictus* were F<sub>1</sub> progeny from a colony collected initially as larvae from tree holes (Indrio Road, Fort Pierce, FL) and propagated as adults in cages (Illinois State University IACUC protocol 01–2005). *C. appendiculata* larvae were from a laboratory colony (generation unknown) maintained since 2005 at the Florida Medical Entomology Laboratory, Vero Beach, FL. The experiment was conducted in a walk-in incubator maintained at 26°C, a photoperiod of 14:10 (L:D) h, and ≈80% humidity.

Behavior of second- and fourth-instar *Ae. albopictus* in control and predation treatments was video recorded using a Panasonic digital video camera (model WV-D5100, Panasonic Corporation of North America, Secaucus, NJ). The control treatment was prepared by holding 10 second-instar *Ae. albopictus* alone, and the predation treatment by holding 10 second-instar *Ae. albopictus* with three fourth-instar *C. appendiculata* for 5 d in 10-ml cups. Dead, eaten, and pupated treatment larvae were replaced daily.

The test larvae were hatched and then held individually in 5 ml of water in 15-ml vials. Each larva was fed 1 ml of liver powder suspension prepared by stirring 0.3 g of liver powder in 1,000 ml water on a stir plate (Juliano and Gravel 2002, Kesavaraju and Juliano 2004). A single feeding was sufficient to rear the 80 *Ae. albopictus* test larvae to second instar, but the 80 fourth-instar test larvae were fed every 2 d until they attained the fourth instar.

Test larvae were starved for 24 h in 10 ml of water before being transferred for behavior recording. Before transfer of test larvae, all predator and prey treatment larvae were removed from the treatment cups, leaving behind only water-borne cues (e.g., uneaten body parts, feces, dissolved chemicals) from the treatment larvae. Depending on the treatment, one second or fourth-instar *Ae. albopictus* was placed in each container and their behavior was recorded using a Winfast XP 2000, TV tuner card (PCI) and associated software (NuMedia Systems USA, Inc. [Leadtek], Walnut, CA) for 15 min. A video clip contained four cups, with one cup for each treatments.

Behaviors were classified into activities and positions in the experimental vessel (Juliano and Reminger 1992). Activities were 1) browsing: mouthparts in contact with the container surfaces; 2) filtering: moving via feeding motions of the mouthparts; 3) thrashing: moving with vigorous lateral flexion of the body; and 4) resting: none of the previous activities. Positions were 1) surface: siphon in contact with water surface; 2) wall: within 1 mm of the sides; 3) bottom: within 1 mm of the bottom; and 4) middle: >1 mm from the sides, bottom, and surface.

Activity and position of the test larvae were noted every 30 s for 15 min upon playback of the video clips. For each behavior, the proportion of the total number of observations ( $n = 30$ ) was determined for each replicate. The number of variables per replicate was reduced by principal components analysis (PCA). Principal components (PC) with eigenvalues >1 were retained and analyzed by a multivariate analysis of variance with the PCs as response variables and the treatments, instars, and interaction as independent variables (Juliano and Gravel 2002, Kesavaraju et al. 2007). Standardized canonical coefficients (SCC) were used to interpret the relative contribution of the PCs in a significant effect (Scheiner 2001).

## Results

There were three PCs with eigenvalues >1 (Table 1). A greater positive score on PC1 indicated that larvae spent more time resting at the surface and a negative score indicated that more time was spent browsing at the wall and bottom. A greater positive score on PC2 indicated that larvae spent more time thrashing in the middle and a negative score indicated more time spent in other behaviors. A greater positive score on PC3 indicated that the larva spent more time

filtering in the middle and a negative score indicated more time spent in other behaviors (Table 1).

The interaction between instar and treatment was significant. SCCs indicated that PC1 contributed most to the significant interaction, followed by PC2 and PC3 (Table 2). Multivariate contrasts revealed that all multivariate means differed significantly except second and fourth instar controls (Table 2). Most importantly second instar responses to predation cues were significantly different from fourth-instar responses to predation cues and second instars showed a much greater change in behavior than did fourth instars (Fig. 1). For all contrasts SCCs indicated that PC1 made the greatest contribution in the significant effect followed by PC2 and PC3 (Table 2).

## Discussion

As predicted, second-instar *Ae. albopictus* reduced their movement at the bottom of containers more than did fourth instar *Ae. albopictus* in the presence of water-borne predation cues from *C. appendiculata* (Fig. 1). Second-instar *Ae. albopictus* are highly vulnerable to predation by *C. appendiculata*, but fourth-instar *Ae. albopictus* are large enough to be relatively invulnerable to predation (Kesavaraju et al. 2007). These results support our hypothesis that behavioral responses of *Ae. albopictus* are proportional to the size-dependent risk of predation. In contrast, another container-dwelling mosquito, *Ae. triseriatus*, showed behavioral responses that were not proportional to the size-dependent threat of predation. Fourth-instar *Ae. triseriatus* are relatively invulnerable to predation by *C. appendiculata* (Kesavaraju et al. 2007), yet fourth-instar *Ae. triseriatus* show essentially the same response to predatory fly larvae as do second instars. Paradoxically, it is the non-native *Ae. albopictus*, which has no evolutionary history with *C. appendiculata* (although they probably encounter other *Corethrella* in their native range in Asia; Miyagi 1974) that shows the predicted behavioral response to size-dependent risk of predation, and not the native *Ae. triseriatus*, which is presumed to have an evolutionary history with *C. appendiculata*.

Whereas the response of *Ae. albopictus* seems to be targeted at a size-selective predator like *C. appendiculata*, the response of *Ae. triseriatus* may not be specific to *C. appendiculata*. *Toxorhynchites rutilus* (Coquillett) larvae, another predator of North American container-dwelling mosquitoes, are larger than *C. appendiculata* larvae, and all instars of *Ae. albopictus* and *Ae. triseriatus* are vulnerable to *Tx. rutilus* predation (Griswold and Lounibos 2005b, 2006). Unlike fourth-instar *Ae. triseriatus*, fourth-instar *Ae. albopictus* do not adopt low risk behaviors similar to the low risk behaviors shown by smaller larvae in the presence of predation risk cues from *Tx. rutilus* (Kesavaraju and Juliano 2004). *Ae. triseriatus* behavioral response to predation risk cues in all stages may represent a general response to cues that could come from multiple predators, and the response by fourth instars would thus help alleviate risk of predation from both *C. appendiculata* and *Tx. rutilus*.

The difference between responses of *Ae. albopictus* and *Ae. triseriatus* to *C. appendiculata* may have consequences for competitive interactions. Antipredatory behavior often comes at a cost of reduced foraging and reduced competitive ability of the prey (Lima and Dill 1990, Sih 1992). For *Ae. albopictus*, the main predator-induced behavioral change is increased resting and reduced browsing (Tables 1 and 2), consistent with a cost of behavioral response. *Ae. albopictus* is superior in resource competition to *Ae. triseriatus* in the absence of *C. appendiculata* (Livdahl and Willey 1991; Novak et al. 1993; Griswold and Lounibos 2005a, b), but in the presence of a limited number of *C. appendiculata*, larvae of the two mosquitoes seem to be able to coexist despite greater predation on *Ae. albopictus* (Griswold and Lounibos 2005a, b). Limited behavioral change by larger stages that are relatively invulnerable to the most abundant predator seems likely to contribute to maintenance of high competitive ability

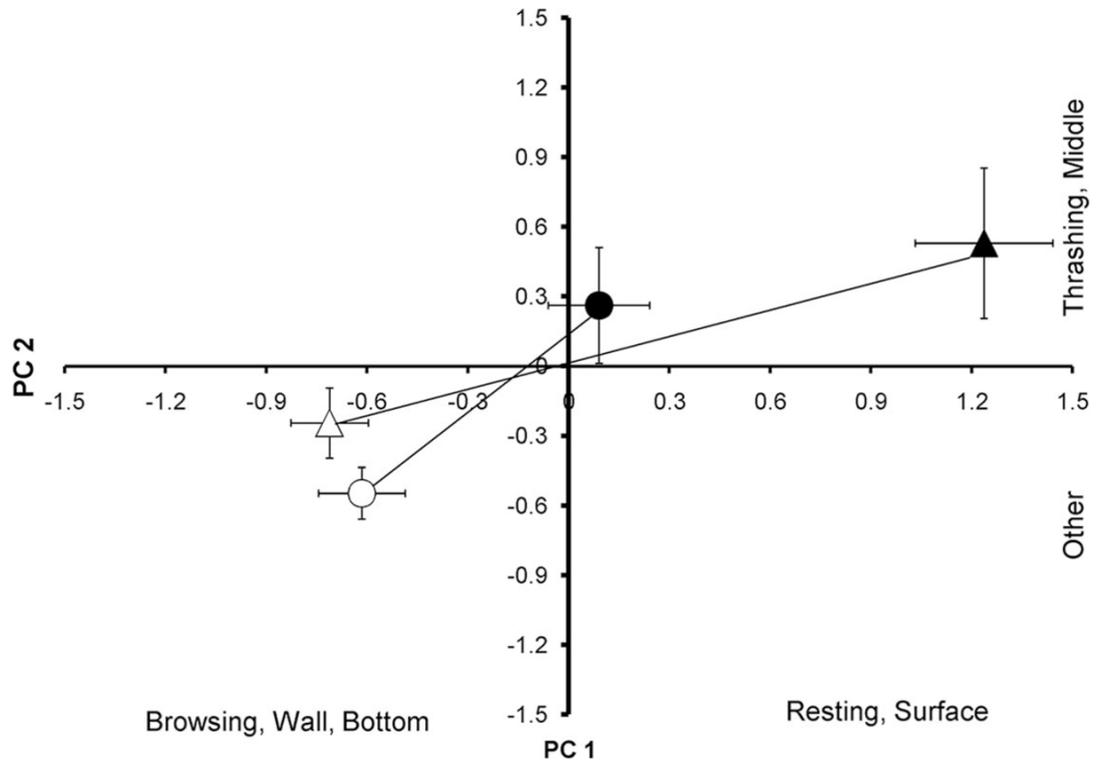
of *Ae. albopictus* even when some *Corethrella* are present. Adopting low risk behaviors in response to water-borne predation risk cues only in the most vulnerable stages may be optimal because such size-dependent responses do not compromise foraging ability in less vulnerable stages.

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**Fig. 1.** Ordination of principal components (means  $\pm$  SE) between second-instar (triangles) and fourth-instar (circles) *Ae. albopictus* in control (open symbols) and predation (closed symbols) treatments ( $N = 30$ ).

**Table 1**Rotated factor patterns of the PCA for comparing the behavioral responses of second and fourth instar *Ae. albopictus*

Variable	PC1	PC2	PC3
Resting	<b>98</b>	9	0
Browsing	<b>-91</b>	-38	-9
Thrashing	24	<b>95</b>	-11
Filtering	2	8	<b>99</b>
Surface	<b>98</b>	9	-1
Wall	<b>-85</b>	-30	1
Middle	26	<b>86</b>	<b>42</b>
Bottom	<b>-65</b>	-36	-21
Interpretation	Resting, surface VS browsing, wall, bottom	Thrashing, middle VS other	Filtering, middle VS other

Values &gt;40 are in bold.

**Table 2**  
Results of MANOVA and multivariate contrasts for the behavioral responses of second and fourth instar *Ae. albopictus*

Variable	Numerator df	Denominator df	Pillai's trace	P	Standardized canonical coefficients		
					PC1	PC2	PC3
Instar	3	62	0.311	<0.0001	1.777	0.906	-0.372
Treatment	3	62	0.725	<0.0001	1.812	0.959	-0.088
Instar × treatment	3	62	0.314	<0.0001	1.757	0.627	-0.499
Multivariate contrasts							
Second instar control vs. fourth instar control	3	62	0.018	0.7744	0.034	0.975	0.465
Fourth instar control vs. fourth instar predation	3	62	0.369	<0.0001	1.623	1.048	0.179
Fourth instar control vs. second instar predation	3	62	0.722	<0.0001	1.817	0.951	-0.172
Second instar control vs. fourth instar predation	3	62	0.332	<0.0001	1.755	0.952	0.106
Second instar control vs. second instar predation	3	62	0.715	<0.0001	1.844	0.884	-0.215
Fourth instar predation vs. second instar predation	3	62	0.471	<0.0001	1.785	0.774	-0.440