

Differential Behavioral Responses to Water-Borne Cues to Predation in Two Container-Dwelling Mosquitoes

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ABSTRACT Larvae of the mosquito *Toxorhynchites rutilus* (Coquillett) prey upon other container-dwelling insects, including larvae of *Aedes albopictus* (Skuse), which is native to Asia but was introduced into the United States, and on the native tree hole mosquito *Ochlerotatus triseriatus* (Say). Previous work has established that *O. triseriatus* adopts low-risk behaviors in the presence of predation risk from *T. rutilus*. It is unknown whether introduced *A. albopictus* show a similar response to this predator. Behavior of fourth instars of *A. albopictus* or *O. triseriatus* was recorded in water that had held either *A. albopictus* or *O. triseriatus* larvae alone (control) and in water that had held *T. rutilus* larvae feeding on either *A. albopictus* or *O. triseriatus* (predation). Activity and position of larvae were recorded in 30-min instantaneous scan censuses. In response to water-borne cues to predation, *O. triseriatus* adopted low-risk behaviors (more resting, less feeding and movement), but *A. albopictus* did not change its behavior. We also tested the species specificity of the cues by recording the behavior of *A. albopictus* in water prepared using *O. triseriatus* and vice versa. *O. triseriatus* adopted low-risk behaviors even in predation water prepared by feeding *T. rutilus* with *A. albopictus*, but *A. albopictus* did not alter its behavior significantly between predation and control treatments prepared using *O. triseriatus*. Thus, *A. albopictus* does not seem to respond behaviorally to cues produced by this predator and may be more vulnerable to predation than is *O. triseriatus*.

KEY WORDS predation risk, *Toxorhynchites*, *Ochlerotatus triseriatus*, *Aedes albopictus*

PREDATION, OR PERCEIVED RISK of predation, induces facultative changes in behavior of mosquito larvae, which can affect their vulnerability to predation (Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002). When larvae are exposed to consistent predation, there can be rapid evolution of these behavioral responses (Juliano and Gravel 2002), suggesting that the responses are adaptive. The Asian container-dwelling mosquito *Aedes albopictus* (Skuse) was introduced into the United States in the mid-1980s and has increased its North American range to include most of the southeastern United States (Hawley et al. 1987, Moore 1999). In southern North America, *A. albopictus* co-occurs in containers with the mosquito *Toxorhynchites rutilus* (Coquillett), which prey on other mosquito larvae, including *A. albopictus* (Campos and Lounibos 2000). *Ochlerotatus triseriatus* (Say), a container-dwelling mosquito native to North America, shows facultative changes in behavior upon perceiving water-borne cues to predation from *T. rutilus* that seem to lessen the risk of predation (Juliano and Reminger 1992, Juliano and Gravel 2002). In mosquitoes, prey behavior strongly affects vulnerability to predation by *Toxorhynchites*

(Rubio et al. 1980, 1981; Juliano and Reminger 1992; Grill and Juliano 1996). There have been numerous studies on the competitive interactions of *A. albopictus* with North American filter-feeding mosquito species such as *Aedes aegypti* (L.) and *O. triseriatus*, and they have shown that *O. triseriatus* is usually an inferior competitor compared with *A. albopictus* (Ho et al. 1989, Livdahl and Willey 1991, Novak et al. 1993, Teng and Apperson 2000), but relatively few studies have been done on the vulnerability of *A. albopictus* to North American predators (Lounibos et al. 2001) or on the impact of predation on the competitive interactions of *A. albopictus* with other species, especially *O. triseriatus*. Because *A. albopictus* is an introduced species that has only recently encountered the predator *T. rutilus*, it is not known whether *A. albopictus* larvae modify their behavior in response to the threat of *T. rutilus* predation. *A. albopictus* does encounter other species of *Toxorhynchites* in its native Asia (Hawley 1988), but there has been no investigation of whether *A. albopictus* modifies behavior in response to these species. *Toxorhynchites* spp. are primarily ambush predators and seem to detect the prey by using mechanoreceptors (Rubio et al. 1980, 1981; Steffan

Table 1. Correlation's of activities and positions for the interspecific comparison experiment

	Thrashing	Browsing	Filtering	Surface	Bottom	Wall	Middle
Resting	-51	-90	-12	93	-36	-76	-40
Thrashing		19	-21	-43	11	24	59
Browsing			-6	-86	31	84	4
Filtering				-14	22	-18	49
Surface					-56	-66	-45
Bottom						-18	29
Wall							-4

All data pooled. Boldfaced numbers represent significant ($P < 0.05$) correlation's.

and Evenhuis 1981). So, highly active prey will likely be more vulnerable to predation compared with less active or resting individuals (Rubio et al. 1980, 1981; Russo 1986; Juliano and Reminger 1992; Grill and Juliano 1996; Juliano and Gravel 2002). Individuals that are highly active can find and harvest more resources and at the same time have more chance of encountering predators (Grill and Juliano 1996). So, behavior, and behavioral change, may play an important role in determining the outcome of both predation and interspecific competition.

We had two specific objectives for this study. First, we wished to determine whether *A. albopictus* shows the same shift to low-risk behaviors upon perceiving water-borne cues to predation that is shown by *O. triseriatus*. *Aedes albopictus* co-occurs with Asian *Toxorhynchites* (Hawley 1988), and if predatory behavior and tactics of those Asian predators are similar to those of North American *T. rutilus*, then the degree of change in behavior should be similar for the two species. Second, we wished to determine whether the water-borne cues from predation that may be perceived by *O. triseriatus* and *A. albopictus* are species specific.

Methods

Comparing Prey Behavior. We collected *O. triseriatus* from tree holes at Parklands Merwin Reserve near Normal, IL, and *A. albopictus* from tires and tree holes at Vero Beach, FL. Both the species were collected as larva and pupae, raised to adulthood in environmental chambers, and propagated in 0.6-m³ cages. *Toxorhynchites rutilus* is relatively rare in Normal (Juliano et al. 1993), so we collected them as larvae in the field at Vero Beach. *Toxorhynchites rutilus*

from this site prey regularly upon *O. triseriatus* (Juliano and Gravel 2002).

The behaviors of *A. albopictus* and *O. triseriatus* larvae were recorded on videotape 1 d after molting to the fourth instar, while they were held in water treated in one of two ways. Control water had held larval conspecifics of each of the species, whereas predation water had held *T. rutilus* feeding on conspecifics of each of the species. For the predation treatment, one *T. rutilus* fourth instar was held for 5 d in a 50-ml cup with 50 ml of water and 10 *O. triseriatus* or *A. albopictus* larvae, depending upon the test species. Larvae offered as prey for water preparation were counted daily and any missing larvae were replaced with additional larvae. For the control treatment, 10 *O. triseriatus* or *A. albopictus* larvae were held 5 d without food. Any larvae that died were replaced. For both predation and control water, some detritus (e.g., feces, bits of eaten prey) accumulated during the 5-d period, and this solid material remained in the treatment water during the trial.

Aedes albopictus and *O. triseriatus* larvae that were used as test subjects were offspring of field-collected individuals. They were hatched and held individually in 18-ml vials with 10 ml of water. These larvae were fed with liver powder suspension (LPS) prepared by mixing 0.3 g of bovine liver powder with 1 liter of water. This food suspension was dispensed via pipetting from a beaker held on a stirring plate to ensure homogeneous delivery of food to larvae (Juliano and Gravel 2002). We provided each larva with 0.5 ml of LPS on day 1 and 1 ml every 2 d thereafter. Once the larvae were fourth instars, they were held individually in 50-ml cups with 30 ml of water and no food for 24 h to standardize hunger before transfer to the treatment water for behavior recording.

Species Specificity of Cues. We prepared the predation water and control water as described in the previous section. We first recorded the behavior of *O. triseriatus* fourth instars in both control and predation water prepared using *O. triseriatus*, and then recorded the behavior of *A. albopictus* fourth instars in the same water. Similarly, we recorded behavior of *A. albopictus* fourth instars in water prepared using *A. albopictus* and then subsequently recorded the behavior of *O. triseriatus* fourth instars in the same water. In each case, test larvae were observed only once, in one kind

Table 2. Principal component analysis for the interspecific comparison experiment

	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	3.92	1.82	1.17	0.87	0.13	0.06
Proportion of variance	0.49	0.22	0.15	0.11	0.02	0.01
Cumulative proportion of variance	0.49	0.72	0.87	0.98	0.99	0.99

Principal components in bold are those with Eigen values >1 that were retained for behavior analysis.

Table 3. Varimax rotated factor pattern for the interspecific comparison experiment

Response variables	PC1	PC2	PC3
Resting	-90	-21	-35
Thrashing	23	-17	93
Browsing	99	07	-03
Filtering	-11	83	-01
Surface	-86	-36	-31
Bottom	25	71	10
Wall	89	-30	02
Middle	02	51	80
Interpretation	Browsing, wall vs. resting, surface	Filtering, bottom, middle vs. other behaviors	Thrashing, middle vs. other behaviors

Values >0.4 are listed in boldface; they indicate strong loadings on each principal component.

of treatment water, but the prepared treatment water was used for one test larva of each species.

Videotaping. We recorded the behaviors of the larvae in the treatment waters on an S-VHS videotape for 30 min. Each larva was given a 10-min acclimation period in the treatment cup before initiating behavior recording. A single 30-min clip had images of a maximum of six treatment cups at a time due to resolution constraints. Each clip had all of the treatments represented.

Observation Protocol. From the videotape, activity and position of the each larva were recorded every minute for 30 min in instantaneous scan censuses (Martin and Bateson 1986, Juliano and Gravel 2002). Activities were classified into four categories: 1) browsing, the larva moving along the surfaces of the cup propelled by feeding movements of the mouthparts; 2) resting, the larva completely still and not feeding; 3) filtering, the larva drifting through the water column, propelled by feeding movements of mouthparts; and 4) thrashing, the larva propelling itself through the water by vigorous lateral flexion of the body (Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002). Positions were classified into four categories: 1) surface, the larva's spiracular siphon in contact with the surface; 2) bottom, the larva within 1 mm of the bottom of the cup; 3) wall, the larva within 1 mm of the sides of the cup; and 4) middle, the larva not in contact with the surface, and >1 mm from the cup's surfaces. For *T. rutilus* preying upon *O. triseriatus*, Juliano and Reminger (1992) showed that among positions, the surface is the least likely to lead to predation, the bottom is the most likely to lead to predation, and middle and wall are intermediate, and among activities, resting is the least likely to lead to predation, thrashing is the most likely

to result in predation, and the two feeding behaviors are intermediate.

Statistical Analysis. We converted the activities and positions to proportions. To reduce the number of variables and to obtain uncorrelated descriptors of behavior, we did principal component analysis on activities and positions (PROC FACTOR, SAS Institute 1990; Juliano and Gravel 2002). Principal components (PCs) with Eigen values >1.0 were retained for further analysis, whereas those with values <1.0 were ignored (Hatcher and Stepansky 1994). Principal component scores were analyzed using multiple analysis of variance (MANOVA) (for comparison of prey behavior) or analysis of variance (ANOVA) (for testing species specificity) (PROC GLM, SAS Institute 1990). We interpreted the results from the MANOVA by using canonical coefficients (Scheiner 2001) that quantify the contributions of the individual principal components in producing significant multivariate differences. We used Tukey-Kramer multiple comparisons between predation and control least-squares means within each species for the behavior experiment. We used Tukey-Kramer multiple comparisons among treatment least-squares means for pairwise comparisons of species specificity treatments.

Results

Comparing Prey Behavior. There were significant positive and negative correlations between position and activity categories. Resting was positively correlated with surface and negatively correlated with thrashing, browsing, and wall (Table 1). Browsing was positively correlated with wall and bottom.

Three PCs summarized 87% of the variation in activity and position (Table 2). Rotated factor pattern

Table 4. MANOVA table for the behavior patterns in the interspecific comparison experiment

Source	df	Den df	Pillai's Trace	P	Standardized canonical coefficients		
					PC1	PC2	PC3
Species (S)	3	56	0.491	<0.0001	0.971	-0.487	0.969
Treatment (T)	3	56	0.461	<0.0001	1.239	0.568	0.575
S * T	3	56	0.178	0.0114	0.876	0.989	0.186

Magnitudes of standardized canonical coefficients indicate the degree of contribution by each factor to the significant MANOVA effect.

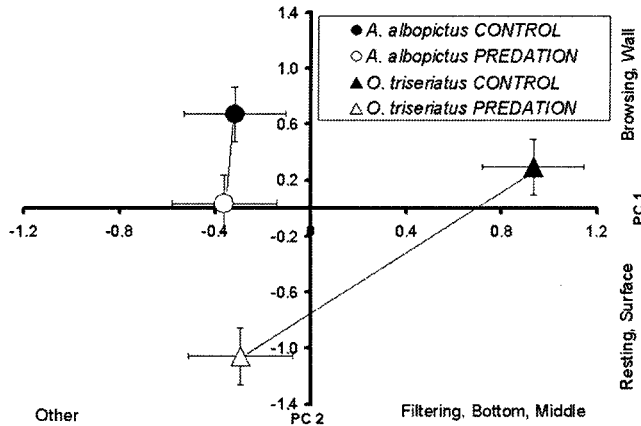


Fig. 1. Plot of PC1 and PC2 (means \pm SE). Activities and positions most closely associated with large positive or large negative PC scores are indicated parallel to each axis. Lines connect means for predation and control treatments for each species. For *A. albopictus*, PC1 and PC2 did not differ significantly between control and predation water treatments (PC1, $P = 0.1181$ and PC2, $P = 0.9989$). For *O. triseriatus*, PC1 and PC2 differed significantly between control and predation water treatments (PC1, $P = 0.0001$ and PC2, $P = 0.0010$) (Tukey–Kramer multiple comparisons).

for PC1 showed large positive coefficients for browsing and wall and large negative coefficients for resting and surface. So, PC1 quantifies allocation time between browsing at the wall and resting at the surface (Table 3). PC2 quantifies time allocation between filtering at the bottom and middle and other behaviors. PC3 quantifies time allocation between thrashing in the middle and other behaviors. Higher PC scores in each case indicate greater frequencies of the activities and positions with large positive factor loadings, and lower frequencies of the activities and positions with large negative factor loadings (highlighted in Table 3).

There was a significant interaction of species and treatment in MANOVA (Table 4), which resulted primarily from scores on PC1 (frequent resting, sur-

face) and PC2 (frequent filtering, bottom, middle). For both PC1 and PC2, behaviors of *A. albopictus* did not differ significantly between control and predation water treatments (PC1, $P = 0.1181$ and PC2, $P = 0.9989$) (Fig. 1). In contrast, *O. triseriatus*'s behaviors differed significantly between control and predation water treatments (PC1, $P = 0.0001$ and PC2, $P = 0.0010$) (Fig. 1). *Ochlerotatus triseriatus* reduced browsing at the wall and filtering and thrashing in the middle considerably in predation water compared with control and increased resting at the surface (Figs. 1 and 2). In predation water, *A. albopictus* did not significantly reduce the frequency of browsing and thrashing in the middle compared with control water (Figs. 1 and 2). PC2 shows that even in the control

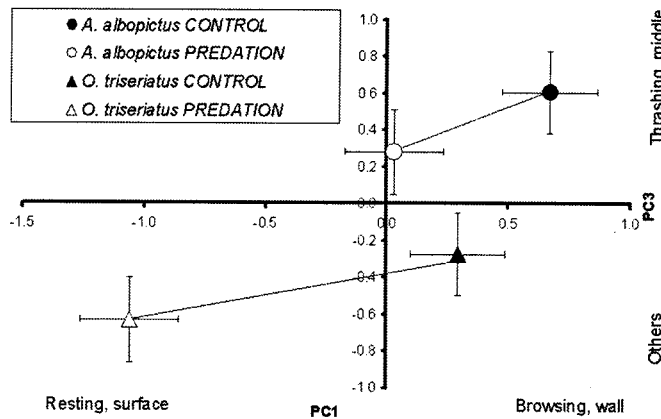


Fig. 2. Plot of PC1 and PC3 (means \pm SE). Activities and positions most closely associated with large positive or large negative PC scores are indicated parallel to each axis. Lines connect means for predation and control treatments for each species. For *A. albopictus*, PC1 and PC3 did not differ significantly between control and predation water treatments (PC1, $P = 0.1181$ and PC3, $P = 0.7423$). For *O. triseriatus*, PC1 differed significantly between control and predation water treatments (PC1, $P = 0.0001$) and PC3 did not differ significantly between control and predation water treatments (PC3, $P = 0.6830$) (Tukey–Kramer multiple comparisons).

Table 5. Correlation's of activities and positions for the species specificity experiment

	Thrashing	Browsing	Filtering	Surface	Bottom	Wall	Middle
Resting	8	-9	15	99	-2	-7	11
Thrashing		26	-10	5	58	17	25
Browsing			25	-8	65	95	25
Filtering				19	25	21	66
Surface					-3	-5	9
Bottom						40	47
Wall							14

All data pooled. Boldfaced numbers represent significant ($P < 0.05$) correlations.

water *A. albopictus* differs considerably in behavior from *O. triseriatus* (Figs. 1 and 2). *A. albopictus* spent less time filtering at the bottom and middle compared with *O. triseriatus*, which allocated more time to filtering at the bottom and middle in the absence of predation risk (Fig. 2).

Species Specificity of Cues. There was a high positive correlation between resting and surface, and browsing was highly correlated with wall and bottom (Table 5). Four of the eight principal components summarized 93% of the variation and had Eigen values >1.0 . The fifth PC was less than one-half of the fourth PC (Table 6). PC2 quantifies resting in surface versus other behaviors. Higher scores on PC2 are associated with more frequent resting in the surface (Table 7). Because resting and surface are the least dangerous activity and position with respect predation (Juliano and Reminger 1992), and because PCs involving resting and surface have been shown to be affected by predator treatments (Juliano and Gravel 2002; this study) we concentrated our analysis on PC2, by using ANOVA. There was a significant test species (*A. albopictus* and *O. triseriatus*) and treatment (control and predation) interaction, indicating that the change in behavior between control and predation water differed significantly between the two test species (Table 8). But there was no three-way interaction (test species, treatment, and preparation) effect, which indicates that the species of prey (conspecific or heterospecific) fed to the *T. rutilus* did not alter the response of the test species to water-borne cues from predation (Table 8). *O. triseriatus* shows a high frequency of low-risk behavior (resting and surface), even in predation water prepared by feeding *A. albopictus* to *T. rutilus* (Fig. 3). In contrast, *A. albopictus* did not alter its behavior in response to any of the treatments (Fig. 3).

Table 6. Principal component analysis for the species specificity experiment

	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	2.89	2.11	1.28	1.13	0.31	0.24
Proportion of variance	0.36	0.26	0.16	0.14	0.04	0.03
Cumulative proportion of variance	0.36	0.63	0.79	0.93	0.97	0.99

Principal components in bold are those with Eigen values >1 that were retained for behavior analysis.

Discussion

Aedes albopictus did not modify behavior in response to water-borne cues from predation by *T. rutilus*, whereas *O. triseriatus* changed its behavior, increasing the frequency of low-risk responses to water-borne cues of predation risk. *Ochlerotatus triseriatus* spent more time resting on the surface in the presence of water-borne cues to predation risk (Fig. 3). Resting on the surface in the presence of predation is the least risky behavior (Juliano and Reminger 1992). In the absence of water-borne cues to predation risk, both *O. triseriatus* and *A. albopictus* spend considerable time browsing along the wall. However, *A. albopictus* does not reduce the risky behavior of thrashing, even in the presence of water-borne cues to predation (Fig. 2). *O. triseriatus* alters its behavior in response to water-borne cues from predation on conspecifics and on *A. albopictus* (Fig. 3). Its response of increased resting at the surface in predation water is thus not species specific to the prey. Its response may thus be a general reaction to cues from any aquatic predator, or alternatively to cues emanating directly from *T. rutilus*.

The absence of a behavioral response in *A. albopictus* may suggest that *A. albopictus* is more vulnerable to predation than *O. triseriatus*. This suggestion is consistent with Campos and Lounibos (2000) who showed that *A. albopictus* is preferred by *T. rutilus* over other prey. Instantaneous risk of mortality due to predation is probably the main variable affected by behavioral changes, but there may be other impacts of a predator such as *T. rutilus* that may complicate assessment of which species is more vulnerable to this predator. *O. triseriatus* seems to have a lower growth rate in the presence of *T. rutilus* compared with *O. triseriatus* that grow in the absence of the *T. rutilus* (Lounibos et al. 1993). This reduced growth is observed even though mortality caused by the predator reduces density of *O. triseriatus*, and should therefore reduce intraspecific competition. It seems likely that low-risk behaviors of resting at the surface have a cost for *O. triseriatus*, probably due to reduced foraging (but see Hechtel and Juliano 1997), that may have an impact on its growth rate. Also, it has been shown that under field conditions *A. albopictus* is a superior competitor to *O. triseriatus* even in the presence of *T. rutilus* (Lounibos et al. 2001). The disadvantage of *O. triseriatus* could be, in part, the result of *O. triseriatus*'s reducing its activity levels in the presence of predation

Table 7. Varimax rotated factor pattern for the species specificity experiment

Response Variables	PC1	PC2	PC3	PC4
Resting	-5	99	6	4
Thrashing	7	6	-5	94
Browsing	95	-6	15	24
Filtering	18	13	91	-15
Surface	-3	100	8	0
Bottom	43	-6	33	73
Wall	98	-2	6	6
Middle	3	3	88	32
Interpretation	Browsing, bottom, wall vs. other behaviors	Resting, surface vs. other behaviors	Filtering, bottom vs. other behaviors	Thrashing, bottom vs. other behaviors

Values >0.4 are listed in boldface; they indicate strong loadings on each principal component.

risk by resting and staying at the surface. In contrast, *A. albopictus* does not adopt these low-risk behaviors and can therefore realize greater feeding and growth rates, and perhaps greater competitive ability. In the absence of predation, growth and development of *A. albopictus* are more rapid than those of *O. triseriatus* (Ho et al. 1989, Livdahl and Willey 1991, Novak et al. 1993), and this slower development may result in the duration of exposure to predation for larval *O. triseriatus* being longer than the corresponding period for *A. albopictus*. Cumulative mortality due to predation is a product of both the instantaneous rate of mortality (presumably lower in *O. triseriatus*) and the duration of exposure (presumably lower for *A. albopictus*), so that prediction of which species will have greater cumulative death rate will be difficult.

Ochlerotatus triseriatus that we used for this experiment were collected from Normal where they seldom encounter *T. rutilus* (Juliano 1996, Juliano and Gravel 2002). Despite this rarity of predation, individuals from this population still alter their behavior in the presence of water-borne cues from this predator (Juliano and Gravel 2002), which suggests that these behavioral responses are an ancestral character for *O. triseriatus*. Juliano and Gravel (2002) showed that when subject to consistent predation by *T. rutilus* in the laboratory, *O. triseriatus* shifted from this facultative response to a constitutive pattern of low movement and resting at the surface. Thus, the facultative response shown by *O. triseriatus* may be most advanced

tageous in situations when the predator is only sometimes present. One interpretation of the absence of any shift in *A. albopictus*, and its generally high level of movement, particularly thrashing (Fig. 2) and browsing (Fig. 1) could therefore be that it has little history of exposure to *Toxorhynchites* predation, and is thus poorly adapted for encounters with this predator. Although in its native range *A. albopictus* occurs in sympatry with several species of *Toxorhynchites* (Hawley 1988) encounters with this group of predators may be rare because of oviposition choices of different-sized containers or of different habitats that may not be preferred by *Toxorhynchites* spp. (Lounibos et al. 2001, Sunahara et al. 2002). Furthermore, North American *A. albopictus* are believed to have originated in temperate Japan (Hawley et al. 1987), and although *Toxorhynchites* occurs in Japan (Collins and Blackwell 2000), this is near its northernmost range limit, hence it may be relatively less common than in tropical Asia. Hence, *A. albopictus* may never have undergone strong selection for behavioral reductions of predation risk. However, the absence of this behavioral response to *T. rutilus* in *A. albopictus* could be explained in several other ways. It remains possible that *A. albopictus* are not using the same kind of cues as *O. triseriatus* to detect predation risk. For example, *A. albopictus* may respond to the visual presence of a predator, or to the combination of visual and water-borne cues. Alternatively, behavioral responses of mosquitoes to predators may be highly predator specific, and *A. albopictus* may not respond to *T. rutilus* because of its lack of evolutionary history with this predator species that is native to North America. In this context, it is important to determine whether *A. albopictus* shows behavioral responses to *Toxorhynchites* species from its native Asia.

Table 8. ANOVA table for PC2 (resting, surface) of the species specificity experiment

Source	df	F	P
Test Species	1	43.89	<0.0001
Treatment	1	25.63	<0.0001
Preparation	1	4.31	0.0525
Test * treatment	1	26.83	<0.0001
Test * preparation	1	6.84	0.0175
Treatment * preparation	1	0.07	0.7944
Test species * treatment * Preparation	1	0.22	0.6427
Error	18		

Test species are *A. albopictus* and *O. triseriatus*. Treatments are control and predation waters.

Preparation indicates whether test water was conditioned using conspecifics or heterospecifics.

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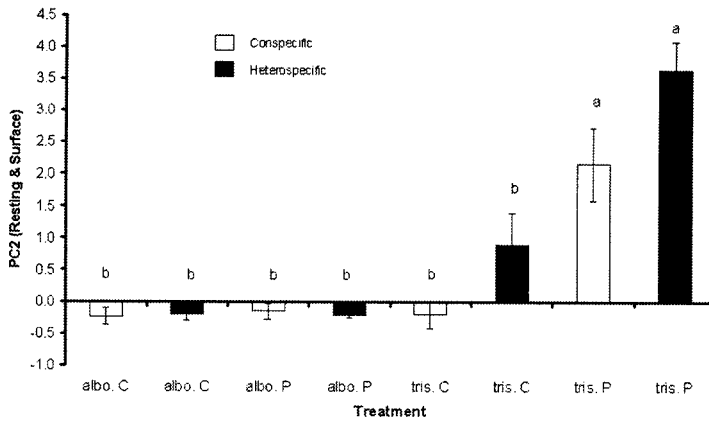


Fig. 3. Species specificity of cues. PC2 (resting at the surface) (mean \pm SE) for all the treatments. albo, *A. albopictus*; tris, *O. triseriatus*; C, control; P, predation; conspecific, water prepared by adding conspecific larvae; heterospecific, water prepared by adding heterospecific larvae (either *O. triseriatus* or *A. albopictus*). Means marked by the same letter are not significantly different (Tukey-Kramer multiple comparisons).

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