

Behavioral Differences of Invasive Container-Dwelling Mosquitoes to a Native Predator

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ABSTRACT Aquatic prey show behavioral modifications in the presence of predation-risk cues that alleviate their risk from predation. *Aedes albopictus* (Skuse), *Aedes japonicus* (Theobald), and *Culex pipiens* L. are invasive mosquitoes in North America, and their larvae are prey for the native mosquito predator, *Toxorhynchites rutilus* (Coquillett). *Ae. albopictus* and *Ae. japonicus* are recent invaders, whereas *Cx. pipiens* has been in the United States for >100 yr. In the presence of predation-risk cues from *Tx. rutilus* larvae, *Cx. pipiens* larvae increased the time spent resting at the surface (least risky behavior) more than the other prey species. *Ae. japonicus* larvae increased resting at the surface of the containers more than *Ae. albopictus* larvae in the presence of predation-risk cues. *Cx. pipiens* larvae spent more time motionless at the surface even in the absence of predation-risk cues when compared with the other species, indicating that *Cx. pipiens* larvae are the least vulnerable prey. As compared with the other prey species, *Ae. albopictus* larvae exhibited more high-risk behaviors both in the presence and absence of predation-risk cues, indicating that they are the most vulnerable prey. *Ae. albopictus* is the superior competitor; however, predation by *Tx. rutilus* larvae may prevent competitive exclusion by *Ae. albopictus* and promote coexistence among the three prey species.

KEY WORDS antipredatory behavior, *Toxorhynchites rutilus*, *Aedes albopictus*, *Aedes japonicus*, *Culex pipiens*

Perception of imminent danger from predators increases prey survival and plays an important role in structuring communities (Lima and Dill 1990). Predation-risk cues are varied, but could be broadly classified into physical and chemical cues. Visual detection of the predator is an example of physical cues, whereas the substances released from conspecifics during predation or from the feces of the predator may function as chemical cues. Predation-risk cues are well studied in aquatic systems where the cues are usually waterborne and chemical in nature (Chivers et al. 2001). Prey respond to the presence of risk in different ways, but behavioral modifications that alleviate the risk of predation are the most common response in aquatic systems (Lima and Dill 1990). Reduction in the level of activity by the prey in the presence of a predator is the most common behavior among antipredatory behavioral modifications (Lima and Dill 1990). Although reduced activity levels benefit the prey by increasing their chances of survival, they also reduce the time spent foraging (Kesavaraju et al. 2007a). Hence, there is often a trade-off between antipredatory behavior and fitness.

Historical predator-prey models have taken into account only casualties from predation. But recent models have shown that responses to the threat of predation can play an important role in survival and fitness of the prey (e.g., Relyea 2000). Wood frog tadpoles grew faster than leopard frogs when they were competing without predation risk, but the outcome of competition was reversed in the presence of caged predators, indicating the importance of trait-mediated indirect effects of predation (Relyea 2000). Because of the trade-off between antipredatory behavior and fitness, some prey species have evolved abilities to judge the quality and quantity of predation risk from these cues (Chivers et al. 2001). Wood frog tadpoles respond within 5 min after being introduced to predator-conditioned water, but do not show any response after 2 h, suggesting that the cues degrade in the environment (Ferrari et al. 2007).

Rainwater that collects in small depressions of trees (tree holes) harbors a specialized insect community dominated by mosquitoes (Kitching 2000). Artificial containers such as cemetery vases and tires that mimic natural containers provide alternative habitats for some mosquito species (Juliano and Lounibos 2005). Some of the common container-dwelling mosquitoes in the eastern regions of the United States are *Aedes albopictus* (Skuse), *Aedes japonicus* (Theobald), and *Culex pipiens* L. The Asian tiger mosquito, *Ae. albopictus*

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tus, is native to southeast Asia and was discovered in the United States in the 1980s (Hawley et al. 1987). Since the initial discovery, it has spread geographically and now occupies most of the Midwest and eastern United States (Juliano and Lounibos 2005). *Ae. albopictus* larvae are superior competitors and have displaced other mosquito species like *Aedes aegypti* (L.) in the southeastern United States (Juliano and Lounibos 2005, O'Meara et al. 1995). *Ae. japonicus* is also native to Asia, was discovered in the northeastern United States in 1999 (Peyton et al. 1999), and has expanded southward. Although *Cx. pipiens* is also an invasive species, it has established in the United States for >100 yr.

The predatory mosquito, *Toxorhynchites rutilus* (Coquillett), commonly occurs in container habitats and preys upon the larvae of *Ae. albopictus*, *Ae. japonicus*, and *Cx. pipiens* (Yee 2008, Farajollahi et al. 2009). The four species commonly co-occur in containers in New Jersey (Farajollahi et al. 2009). Larvae of *Tx. rutilus* are voracious and highly cannibalistic; consequently, only a single larva usually survives to the pupal stage in each container (Steffan and Evenhuis 1981). *Tx. rutilus* larvae use mechanoreceptors to detect the presence of prey and primarily hunt at the bottom of the containers. Mosquito larvae that move more frequently at the bottom of the containers are at a higher risk of being captured by these predators than are those larvae that rest motionless at the surface (Juliano and Reminger 1992).

Aedes triseriatus (Say) is a container-dwelling mosquito native to the United States with a long evolutionary history with *Tx. rutilus*. *Ae. triseriatus* larvae reduce their movements at the bottom of the containers in the presence of predation-risk cues from *Tx. rutilus* (Kesavaraju and Juliano 2004). This reduction in movement at the bottom of the containers has been shown to reduce the probability of being captured by *Tx. rutilus* (Juliano and Reminger 1992). Unlike *Ae. triseriatus*, *Ae. albopictus* larvae do not show any changes in behavior when predator cues are present in container habitats (Kesavaraju and Juliano 2004). *Ae. japonicus* also has a very brief history with the native predator because of recent invasion, but it is not known whether larvae exhibit behavioral responses to chemical cues from *Tx. rutilus*. Larvae of *Cx. pipiens* modify their behavior in the physical presence of notonectids, an aquatic insect predator (Sih 1984), but it is not known whether *Cx. pipiens* larvae can detect the waterborne cues from *Tx. rutilus* larvae.

The interaction between competitive ability and susceptibility to predation influences the geographic distribution of container-dwelling mosquitoes. Andreadis and Wolfe (2010) reported a 90% reduction in container mosquitoes such as *Ae. triseriatus* after *Ae. japonicus* invaded the northeastern United States. *Cx. pipiens* is widespread across the northern regions of the United States, and larvae co-occur and compete with *Ae. japonicus* and *Ae. albopictus*. *Ae. albopictus* is competitively superior to *Ae. japonicus* (Armistead et al. 2008) and *Cx. pipiens* (Costanzo et al. 2005). Prior studies showed that *Ae. albopictus* can displace other

Aedes in many areas in the southeastern United States (O'Meara et al. 1995, Juliano and Lounibos 2005). The outcome of competition between *Ae. japonicus* and *Cx. pipiens* has not been studied, so it is difficult to predict the outcome of competitive interactions in areas where they co-occur without *Ae. albopictus*.

The objective of our research was to compare the behavior of *Ae. albopictus*, *Cx. pipiens*, and *Ae. japonicus* larvae in the presence and absence of predation-risk cues from *Tx. rutilus* larvae.

Materials and Methods

Larvae of *Ae. albopictus* were collected from cemetery vases and discarded tires in Mercer County, New Jersey. Adults were held in 30 × 30-cm cages and provided with 10% sucrose solution, and females were allowed to blood feed on restrained quails. Eggs of *Ae. japonicus* and *Cx. pipiens* were obtained from a laboratory colony maintained at the Center for Vector Biology (Rutgers University, NJ). Larvae of *Tx. rutilus* were obtained from a laboratory colony established from field-collected larvae from New Jersey.

Larval Rearing. Filter papers containing eggs of *Ae. albopictus* and *Ae. japonicus* were immersed for 24 h in a hatching medium consisting of 1,000 ml of deionized water and 0.15 g of Brewers yeast (B):lactalbumin (L) (50:50). Egg rafts of *Cx. pipiens* were left on the hatching medium for 48 h. First instars were isolated and placed in individual 10-ml screw top vials (without the cap) with 3 ml of water. Larvae were fed with 1 ml of B:L suspension prepared by stirring 0.15 g of B:L in 1,000 ml of water on a stir plate. Larvae were fed on the fourth day and then every other day until the eighth day for *Ae. albopictus* and until the 10th day for *Cx. pipiens* and *Ae. japonicus*. Larvae were held in 100-ml polystyrene cups without food for 24 h to standardize hunger before being transferred to the treatment water.

Treatment Water Preparation. Predation-risk cues (predation water) were prepared by holding 10 fourth-instar *Cx. pipiens*, *Ae. albopictus*, or *Ae. japonicus* with one third-instar *Tx. rutilus* for 4 d in 100-ml polystyrene cups with 75 ml of deionized water. Cups were checked daily, and dead, eaten, or pupated larvae were replaced. Treatment water was species specific, that is *Cx. pipiens* behavior was recorded only in predation-conditioned water prepared by feeding *Tx. rutilus* with *Cx. pipiens*. Control water was prepared similarly to predation water, except that *Tx. rutilus* was not added to the cups.

Behavior Recording. Behavior of *Cx. pipiens*, *Ae. albopictus*, and *Ae. japonicus* larvae was studied by placing a single larva of each species into a predation or control treatment. Before the focal larva was introduced into the test environment, the mosquitoes used to condition the water were removed from the experimental vessels. Larval behavior was recorded onto a hard disk drive using a Sony HD 40 GB Handycam camcorder, and each video clip contained six cups (three predation and three controls representing each species). The larvae were allowed to

acclimate for 5 min after they were transferred to the experimental vessel before recording started.

The activity and position of each larva were recorded every minute for 30 min with instantaneous scan censuses (Juliano and Reminger 1992). Activity was classified into four types, as follows: resting (when the larva was not moving through the water column), thrashing (when the larva was moving with vigorous side to side flexions of the body), browsing (larval mouthparts were in contact with the surface of the container), and filtering (the larva was moving with the help of its mouth parts along the water column). Four positions were also classified following Juliano and Reminger (1992): surface (the siphon was in contact with surface of the water column), wall (larva was within 1 mm from the sides of the container), bottom (larva was within 1 mm from the bottom of the container), and middle (larva was >1 mm from the sides and bottom of the container). Only one activity and one position were recorded with an instantaneous scan every minute for 30 min, resulting in 30 activities and 30 positions for each replicate. Resting and surface are the least risky activity and position, whereas thrashing and bottom are most risky behaviors (Juliano and Reminger 1992) when a *Tx. rutilus* larva is present.

Statistical Analysis. Activities and positions were converted to proportions based on the 30 observations for each replicate, resulting in eight response variables (Juliano and Reminger 1992, Juliano and Gravel 2002). Principal component (PC) analysis was used to reduce the number of variables. PC that had eigenvalues >1 were retained and analyzed with a multivariate analysis of variance (Juliano and Reminger 1992, Kesavaraju and Juliano 2004). Standardized canonical coefficients were used to determine the relative contributions of the PCs when behavior differed significantly among the mosquito species (Scheiner 2001).

Results

PC analysis resulted in three PCs that had eigenvalues >1, and those PCs explained 90% of the variation in larval mosquito behavior. Larvae that exhibited a large negative score on PC1 (eigenvalue = 4.48, variation explained = 55%) spent more time resting on the surface, whereas a high positive score indicated that larvae spent more time thrashing in the middle. A large negative score on PC2 (eigenvalue = 1.58, variation explained = 20%) indicated that larvae spent more time resting and browsing at the surface, and a high positive score meant larvae spent more time near the wall. A negative score on PC3 (eigenvalue = 1.11, variation explained = 15%) meant larvae spent more time filtering in the middle of the container, and a positive score meant that larvae spent more time at the bottom of the containers than at the other positions (Table 1).

The interaction between treatment and species was significant, indicating that the behaviors of the species were different between treatment waters (Table 2). Standardized canonical coefficients showed that a

Table 1. Rotated factor patterns for the principal components

Variables	Principal components		
	PC1	PC2	PC3
Resting	-57 ^a	-66	-39
Browsing	25	-77	5
Thrashing	90	21	10
Filtering	39	-16	-69
Surface	-47	-67	-55
Wall	9	97	-14
Bottom	33	0	90
Middle	94	21	-1
Interpretation	Resting, surface VS thrashing, middle	Resting, browsing, surface VS wall	Filtering, surface VS bottom

^a Numbers >40 were used for interpreting PCs.

positive correlation from PC1 and a negative correlation from PC2 were mostly responsible for the significant effect. Multivariate contrasts revealed that all possible comparisons were statistically different. The behavior of the three species was different between and within control and predation treatments. Standardized canonical coefficients showed that PC1 and PC2 contributed more to the significant differences than did PC3 (Table 3).

All species changed their behavior in the predation treatment by increasing the frequency of resting near the surface. Regardless of water treatment, *Cx. pipiens* larvae rested more often near the surface of the water than did the other two species (Figs. 1 and 2). Multivariate contrasts of behavior between control and predation treatments (same species) indicated that PC1 primarily accounted for the significant effect for *Cx. pipiens* (Table 3). PC1 represents the difference between resting at the surface (least risky behaviors) and thrashing (most risky behavior). *Cx. pipiens* larvae are least at risk from predation by *Tx. rutilus*. *Ae. japonicus* larvae spent more time browsing or thrashing near the wall, middle, and bottom of the container in control treatments, but reduced their movements and increased resting near the surface in the presence of predation-risk cues. *Ae. albopictus* larvae browsed near the wall and at the bottom of containers more than the other activities and positions in control treatment, but increased thrashing in the middle of the container and resting near the surface in the presence of predation cues (Figs. 1 and 2).

Discussion

All of the mosquitoes modified their behavior in the presence of predation-risk cues from *Tx. rutilus*. However, *Cx. pipiens* larvae increased the time spent resting at the surface more so than did *Ae. albopictus* or *Ae. japonicus* larvae. Larvae of *Cx. pipiens* rested more often near the surface of the containers even in the control water when compared with other species, indicating that they spend less time moving in the containers. Larvae of *Ae. albopictus* exhibited more risky behaviors than did the larvae of the other species regardless of treatment type, indicating that they will

Table 2. Multivariate analysis of variance results on the PCs of behavioral comparison between *Ae. albopictus*, *Ae. japonicus*, and *Cx. pipiens* in response to *Tx. rutilus* predation cues

Variables	Num DF	Den DF	Pillai's trace	P	Standardized canonical coefficients		
					PC1	PC2	PC3
Treatment (T)	3	108	13.28	<0.0001	0.9404	1.9718	0.5249
Species (S)	6	218	39.96	<0.0001	1.0221	1.9018	0.7317
T × S	6	218	2.41	0.0284	0.8544	-0.6754	-0.3451

Num DF, numerator degrees of freedom; Den DF, denominator degrees of freedom.

be most vulnerable to predation from *Tx. rutilus*. Kesavaraju and Juliano (2004) concluded that *Ae. albopictus* do not show any behavioral modifications when compared with *Ae. triseriatus* in the presence of *Tx. rutilus* cues. Although the treatment for *Ae. albopictus* was not significantly different, there was a pattern of change in behavior between control and predation. Larvae of *Ae. albopictus* in that study altered their behaviors in predation treatment compared with control, but multiple comparisons indicated that there was no statistically significant difference (Kesavaraju and Juliano 2004). Alternatively, in our study, there is a strong and significant pattern of change in behavior, but the change in the presence of predation risk is not as much toward the low-risk behaviors compared with other species. Sih (1984) reported that *Cx. pipiens* larvae reduced their movement in the presence of the predatory notonectids and avoided areas frequented by them in containers. Our results are similar to Sih's, but also suggest that *Cx. pipiens* larvae can detect the waterborne cues from predators and/or resulting from predation of conspecific larvae.

The nature of chemical predation-risk cues has been well studied in ostariophysian fishes. Chivers et al. (2007) showed that the alarm cues to which minnows respond are released by epidermal cells that have an immune function, but are damaged at the site of an attack by a predator. Without structural damage, these epidermal cells do not release the chemicals that serve as the alarm cue even when predators are present. In container mosquito communities, the amount of water in the habitats is often <1 liter; hence, the nature of predation-risk cues could be different from larger habitats. Larvae of *Ae. triseriatus* showed no behavioral response to a caged nonfeeding *Tx. rutilus* (Hechtel and Juliano 1997), but when the cues were

filtered and separated into liquid and solid (filtrate) *Ae. triseriatus* larvae showed more antipredatory behavioral modifications to the presence of solid cues (Kesavaraju and Juliano 2010). Larvae of *Cx. pipiens* and *Ae. japonicus* could be using similar cues to detect the presence of predation risk.

Cx. pipiens larvae increased their resting at the surface in the presence of predation-risk cues, which reduces the time spent in foraging activities. Beketov and Liess (2007) showed that *Cx. pipiens* larvae reduced growth and survival when they were reared in water conditioned by a feeding notonectid compared with those that were reared with conspecifics. Because both the direct and indirect effects of predation significantly affect the survival of larvae, evolutionary responses should favor adult females that can detect the presence of predation-risk cues and avoid depositing eggs in those habitats. Adults of *Culiseta longiareolata* (Macquart) can sense an airborne kairomone to avoid habitats with predators and do not need to land on the water surface to detect the presence of predation risk (Silberbush and Blaustein 2008). Several *Culex* mosquitoes avoid ovipositing in pools that have the aquatic predator *Notonecta irrorata* (Uhler) and continue to avoid them for ~2 wk, even those pools that formerly had *N. irrorata* (Blaustein and Chase 2005). Adult females of the mosquito *Culex tarsalis* (Coquillett) avoid pools that have predation-risk cues from larvivorous fish, such as the mosquitofish *Gambusia affinis* (Baird and Girard) (Walton et al. 2009). Angelon and Petranka (2002) showed the *Cx. pipiens* adults also avoid pools that have predation-risk cues from *G. affinis*. So it remains possible that *Cx. pipiens* adults can sense the presence of *Tx. rutilus* in containers and avoid oviposition in those containers.

Although it is possible that they encounter other larval predators in their native Asian range, *Ae. ja-*

Table 3. Multivariate contrasts of the PCs for the behavioral comparison experiment

Variables	Num DF	Den DF	Pillai's trace	P	Standardized canonical coefficients		
					PC1	PC2	PC3
<i>Ae. albopictus</i> control vs <i>Ae. albopictus</i> predation	3	108	0.15	<0.0005	0.2279	1.8198	0.6885
<i>Cx. pipiens</i> control vs <i>Cx. pipiens</i> predation	3	108	0.11	<0.0061	1.4093	1.134	0.3169
<i>Ae. japonicus</i> control vs <i>Ae. japonicus</i> predation	3	108	0.15	0.0006	0.8328	1.9346	0.2469
<i>Ae. albopictus</i> control vs <i>Cx. pipiens</i> control	3	108	0.67	<0.0001	0.7543	1.9774	0.718
<i>Ae. albopictus</i> control vs <i>Ae. japonicus</i> control	3	108	0.23	<0.0001	-0.2887	1.5172	0.2687
<i>Ae. japonicus</i> control vs <i>Cx. pipiens</i> control	3	108	0.56	<0.0001	1.0909	1.8231	0.7907
<i>Ae. albopictus</i> predation vs <i>Cx. pipiens</i> predation	3	108	0.65	<0.0001	1.1136	1.8661	0.6439
<i>Ae. albopictus</i> predation vs <i>Ae. japonicus</i> predation	3	108	0.19	<0.0001	0.1857	1.6958	-0.0918
<i>Ae. japonicus</i> predation vs <i>Cx. pipiens</i> predation	3	108	0.55	<0.0001	1.2706	1.5379	0.8197

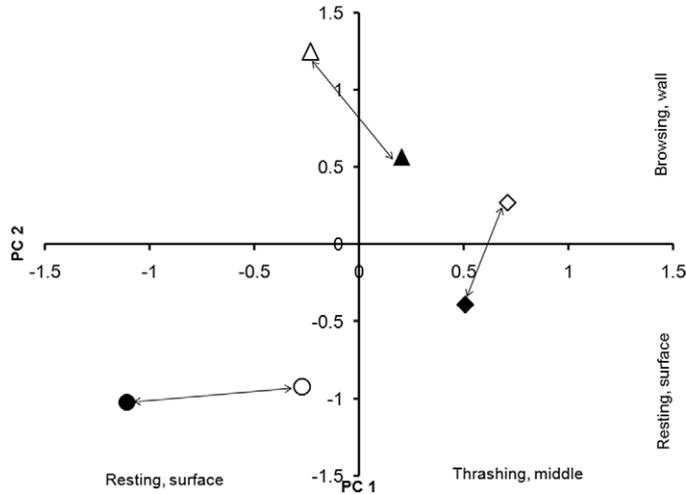


Fig. 1. Bivariate plots of PC1 and PC2 (mean \pm SE) for the behavioral comparison. Triangles are *Ae. albopictus*, circles are *Cx. pipiens*, and diamonds are *Ae. japonicus*. Open symbols are control, and closed symbols are predation. Error bars are small, and hence are not visible.

ponicus are recent invaders and have had relatively less evolutionary time with *Tx. rutilus*. Larvae of *Ae. triseriatus* showed behavioral changes after two generations of selection by *Tx. rutilus* predation by reducing their movements even in control water treatments (Juliano and Gravel 2002). Alternatively, *Ae. albopictus* showed no evolutionary response after four generations of selection by *Corethrella appendiculata* (Grabham) predation (Kesavaraju and Juliano 2009). *Ae. triseriatus* collected from areas where *Tx. rutilus* larvae were common showed more lower predation-risk behaviors compared with larvae collected from areas where *Tx. rutilus* were rare. Alternatively, *Ae. albopictus* populations from areas with variable *C. appendiculata* abundances did not show significant vari-

ation in low-risk behaviors. Further research is needed to elucidate the evolutionary potential of antipredatory behaviors in *Ae. japonicus* (Kesavaraju and Juliano 2009). *Ae. japonicus* that we used in experiments were from a laboratory colony, and, hence, it is probable that they differ in behavior from wild-caught individuals. *Cx. pipiens* that we used in the experiments were also from a laboratory colony. Several generations of laboratory selection apparently did not eliminate antipredatory behavioral modifications.

The importance of antipredatory behaviors in mosquitoes has been well documented in the container systems. Kesavaraju et al. (2008) showed that behavioral responses of container-dwelling mosquitoes to predators can influence the community composition

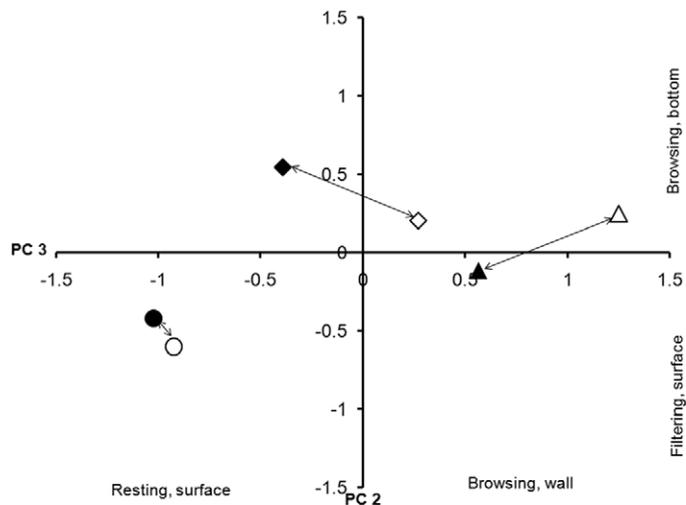


Fig. 2. Bivariate plots of PC2 and PC3 (mean \pm SE) for the behavioral comparison. Triangles are *Ae. albopictus*, circles are *Cx. pipiens*, and diamonds are *Ae. japonicus*. Open symbols are control, and closed symbols are predation. Error bars are small, and hence are not visible.

and, in turn, the distribution of mosquitoes. Larvae of *C. appendiculata* prey on second instars of *Ae. triseriatus* and *Ae. albopictus* (Kesavaraju et al. 2007b). *C. appendiculata* larvae use mechanoreceptors and hunt primarily at the bottom of the containers, similar to *Tx. rutilus*. *Ae. triseriatus* larvae modify their behavior by reducing the time spent at the bottom and increasing the time spent near the surface of the containers. *Ae. albopictus* show similar behavioral responses, but are more active than *Ae. triseriatus* larvae and are more vulnerable to predation by *C. appendiculata* (Kesavaraju et al. 2007b). *Ae. albopictus* is a superior competitor to *Ae. triseriatus* under laboratory conditions. Kesavaraju et al. (2008) showed that the abundance of *C. appendiculata* larvae is positively correlated with *Ae. triseriatus* abundance and negatively correlated with *Ae. albopictus* abundance, especially in tree hole habitats where *C. appendiculata* larvae are more common. Also, the abundance of *Ae. albopictus* in artificial container habitats where *C. appendiculata* are less common was twice that in natural container habitats like tree holes. Accordingly, Kesavaraju et al. (2008) concluded that *C. appendiculata* may be a keystone species inhibiting competitive exclusion of congeners by *Ae. albopictus*. Similarly, *Tx. rutilus* could be a keystone species facilitating coexistence of *Ae. albopictus*, *Ae. japonicus*, and *Cx. pipiens* in areas where they are abundant. Future research on competitive interactions between the three species with or without the presence of *Tx. rutilus* would provide more information on the subject.

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