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Predation resistance does not trade off with competitive ability in early-colonizing mosquitoes

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Abstract

The tradeoff between colonization and competitive ability has been proposed as a mechanism for ecological succession, and this tradeoff has been demonstrated in multiple successional communities. The tradeoff between competitive ability and predation resistance is also a widely-described phenomenon; however, this tradeoff is not usually postulated as a cause of ecological succession. Early successional species that arrive before predator colonization could be either (1) Less vulnerable to predation than their successors, by virtue of being poor competitors (direct competition-predation tradeoff); (2) Equally or more vulnerable to predation, because they normally colonize ahead of predators in succession and therefore are not evolutionarily adapted to avoid predators that they rarely encounter (no competition-predation tradeoff). To test these alternative hypotheses, we established water-filled containers in an oak-hickory forest. We allowed half of the containers to be naturally colonized by early-successional *Culex* mosquitoes, mid-successional *Aedes* mosquitoes, and the mosquito predator *Toxorhynchites rutilus*. In the other half of the containers we prevented *Aedes* colonization via systematic removal of *Aedes* eggs, but allowed *Culex* and *T. rutilus* to colonize. The numbers of mature *Culex* larvae and pupae, and later the total number of *Culex*, were significantly greater in containers where *Aedes* had been removed, which suggests that *Culex* are competitively suppressed by *Aedes*. *Toxorhynchites rutilus* abundance and colonization rate were unaffected by the removal of *Aedes*, and densities of both *Culex* and *Aedes* decreased significantly with *T. rutilus* abundance in both treatments. In-lab bioassays showed that *Culex* were significantly more vulnerable to predation by *T. rutilus* than were *Aedes*. These data are consistent with the hypothesis that *Culex* and *Aedes* demonstrate a direct colonization/competition tradeoff, and are inconsistent with the hypothesis of a direct competition/predation tradeoff.

Keywords

Aedes; colonization-competition tradeoff; competition-predation tradeoff; *Culex*; mosquitoes; succession

Introduction

Niche tradeoffs in species occurring along an environmental or biotic gradient are thought to promote diversity in ecological communities (Chase and Leibold 2003, McCook 1994). One commonly cited tradeoff is between colonization ability and competitive ability of species. In the context of ecological succession, early successional species are postulated to be

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adapted for dispersal and colonization of new, uncolonized habitats and generally poor resource competitors (Tilman 1994, Connell and Slatyer 1977). Species that are less rapid colonizers, on the other hand, should be better resource competitors, which ultimately enables them to colonize, and eventually to dominate, habitats in which early colonizers are already abundant (Tilman 1994, Connell and Slatyer 1977). This tradeoff has been demonstrated in laboratory experiments with protozoans (Cadotte et al. 2006) and fungi (Kennedy et al. 2011), and in a field study with terrestrial plants (Gleeson and Tilman 1994).

Another common tradeoff is that between competitive ability and predator vulnerability of species. Organisms that invest more in the acquisition of resources may invest less in defense mechanisms, therefore making competitive organisms more vulnerable to predation (Leibold 1996). This tradeoff can take a number of forms. In plant communities, more competitive species are often defined as those species with faster growth at low resource levels, higher proportion of biomass allocated to roots, or greater leaf surface in proportion to biomass (Godoy et al. 2011). Less competitive plant species are predicted to allocate resources instead to greater chemical or physical defenses against herbivory (Viola et al. 2010). In animal species, greater competitive ability is generally associated with greater foraging effort, movement, and more rapid growth at low food availability (Wellborn et al. 1996, Wissinger et al. 1999, Kesavaraju et al. 2008). However, this greater foraging effort and movement often attracts the attention of predators and lowers the predator vigilance of the prey species (Wissinger et al. 1999, Kesavaraju et al. 2008), thus making these superior competitors more susceptible to predation.

While such a tradeoff is logical given a homogeneous environment, it is possible for such tradeoffs to be confounded with other tradeoffs, particularly during ecological succession. Colonizing species often must be adapted to the dual threats of competition and predation simultaneously (Tirok and Gaedke 2010), either by developing tolerance to the predator, increasing competitive ability, or avoiding one or both of these ecological threats by colonizing unoccupied habitat (Wellborn et al. 1996). But, if a species is able to colonize and to gain reproductive success in habitats prior to the arrival of predators, would this species be more, or less vulnerable to predation than later colonizers? On the one hand, an early colonizer that is a poor competitor might also be less vulnerable to predation because of the tradeoff between competitive ability and predator vulnerability (Wissinger et al. 1999, Kesavaraju et al. 2008, Viola et al. 2010). On the other hand, an early colonizer that occupies a habitat prior to colonization by predators may be both a poor competitor and predator vulnerable, as it has adapted to exploit environments with both low predation and low competition.

A good system in which to test the tradeoffs among colonization, competition, and predator vulnerability is the aquatic community of larval Diptera that occurs in water-filled containers. In the North American Midwest, these communities are dominated by three taxa of mosquitoes: *Culex* (including *Culex pipiens*, *Culex quinquefasciatus*, *Culex restuans*, and *Culex territans*), *Aedes* (including *Aedes japonicus*, *Aedes triseriatus*, and *Aedes albopictus*), and *Toxorhynchites rutilus*. Both *Culex* and *Aedes* larvae are detritivorous, browsing and filter-feeding on microorganisms growing on decaying leaf and animal detritus (Juliano 2009). *Toxorhynchites rutilus* larvae are generalist predators that feed upon both *Culex* and *Aedes* (Yee 2008, Griswold and Lounibos 2006). Previous laboratory studies have shown that *Aedes* are consistently superior resource competitors to *Culex* (Carrieri et al. 2003, Costanzo et al. 2005a, Murrell and Juliano 2012). Predator vulnerabilities of the two genera are less well known; although *C. pipiens* has been shown to be less vulnerable to predation than *A. albopictus* (Farajollahi et al. 2009), the predator vulnerabilities of *C. restuans*, *A. japonicus*, and *A. triseriatus* in relation to each other are unknown.

Furthermore, relative predator impacts on populations of members of these genera have not been tested via manipulations in the field.

Fortunately, a life history characteristic of *Aedes* mosquitoes provides a mechanism by which we can experimentally test the tradeoffs among colonization, competition, and predation resistance in a field setting. Unlike *Culex* and *Toxorhynchites*, which oviposit directly on the surface of the water within a container, *Aedes* oviposit on the inside wall of the container, just above the surface of the water (Bentley and Day 1989). *Aedes* eggs typically do not hatch until the container fills with more water and floods the eggs (Bentley and Day 1989). By removing freshly laid *Aedes* eggs from a container on a regular basis, one can effectively prevent *Aedes* from colonizing a container without directly altering colonization by either *Culex* or *Toxorhynchites*.

We used this life history characteristic to remove *Aedes*, a highly competitive genus in these container systems (Juliano 2009) from the community, to test the following hypotheses: (1) Early-colonizing *Culex* decline because of competition from later-colonizing *Aedes* (i.e., colonization/competition tradeoff), (2) *Culex* are less vulnerable to predation than *Aedes* (i.e., direct competition/predation tradeoff). The first hypothesis predicts that removal of *Aedes* from containers should result in increased number of *Culex* or number of mature *Culex* (e.g., 4th instar larvae and pupae) when compared to containers where *Aedes* are allowed to colonize. The second hypothesis predicts that removal of *Aedes* should cause either a delay in the colonization of *T. rutilus*, or a decrease in *T. rutilus* abundance, as a result of removing their primary food source (*Aedes*).

METHODS

Colonization Data

Eighty 5-gallon black buckets were established in pairs (1 control, 1 removal treatment container) along a forested road at Tyson Research Center, Eureka, MO, on June 2 - 3, 2010. The buckets within a pair were set at least 1 m apart; each pair was placed 10 m from the center of the road and 20 m from other pairs of buckets on the same side of the road. The top 20 cm of all buckets was lined with white enamel-coated aluminum sheeting. This sheeting provided a substrate to which *Aedes* eggs adhered; however, once laid these black eggs were visible to the naked eye. The lining was secured to the buckets using binder clips, to prevent insects from ovipositing between the lining and the side of the bucket.

On June 3 - 4, each bucket was filled with 15 L well water from Tyson's water supply, 30 g of dried white oak leaves, and 100 mL hay infusion (50 g/L) as an inoculum. Every 2 days, the sheeting from each container was removed and assessed for the presence of mosquito eggs. For the removal treatment containers, all mosquito eggs were removed from the sheet, and the sheet was placed back in the container. For control containers, the sheet was checked for eggs, and then replaced in the container without any egg removal.

Beginning June 10, containers were sampled weekly for 7 weeks for both predator and prey colonization. To assess predator colonization, we ran a sieve (20.3 cm diameter, 106 μ m mesh) quickly through the bucket twice, then dumped the contents of the sieve into a white pan, counted the number of *T. rutilus* present, determined the stages of the *T. rutilus* (1st instar - pupa), and returned all *T. rutilus* to the container. To sample prey species, we placed a 90-lb-pull, gasket-lined magnet into the container, allowed 3 minutes for the disturbed community to resettle, then quickly plunged a 6.4 cm-diameter steel tube into the container and onto the magnet. The magnet adhered tightly to the end of the tube, sealing in a sample of the water column containing both pelagic and benthic species. The mean volume of this sample was 627.8 ± 3.4 mL (approximately 5% of the total volume of the bucket). Buckets

received natural inputs of rain, and volumes were roughly stable over the summer. Any *T. rutilus* captured in the sample were returned to the container to prevent consumption of prey larvae in the sample. The prey larvae were then bottled, identified in the laboratory, and then preserved in 70% ethanol. To determine the developmental stages of *Culex* and *Aedes* in each sample, we counted the number that were pupae, 4th, or 3rd instar larvae in the preserved samples, and determined the numbers of 1st and 2nd instar larvae by subtraction from the total counts of immatures. Instar was determined by the size of the head capsule, which is a reliable indicator of larval stage for both *Aedes* (Alto et al. 2009) and *Culex* (Rueda et al. 1990).

Assessment of Prey Vulnerability

We conducted an in-lab bioassay during the summer of 2011 to determine vulnerability of *C. pipiens*, *C. restuans*, *A. japonicus*, and *A. triseriatus* to predation by *T. rutilus*. Twenty 4th instar larvae of a single species were collected from field containers and transferred to a small plastic beaker filled with 20 mL nanopure water. Two 3 cm diameter circles of paper towel were placed in the bottom of the container to simulate spatial heterogeneity provided by leaf litter. A single 4th instar *T. rutilus* larvae, which had been starved for 24 hours, was then placed in the beaker. Each day we counted the number of prey killed or consumed by *T. rutilus*, removed and discarded pupae and undamaged dead prey, and replenished the number of prey larvae to 20 individuals. We repeated this for four days, after which we calculated the cumulative number of prey eaten per *T. rutilus*. Any replicates in which the *T. rutilus* larva pupated in less than 4 days were excluded from the analysis. Between 4–9 replicates were collected for each prey species.

Statistical Analyses

For *Culex* and *Aedes* abundances, we conducted separate repeated-measures mixed model analyses with a Poisson distribution (PROC GLIMMIX, SAS 9.1) to test for simultaneous effects of *Aedes* removal and *T. rutilus* abundance on week's 4–7 of colonization data. Prior to week 4, *T. rutilus* did not colonize containers and *Aedes* colonization was very low (often 0; averaging <1 larva/sample, or approximately <22 larvae/container); therefore, it was impossible to test the effects of *T. rutilus* abundance and *Aedes* removal for all 7 weeks. Significant effects of week, treatment (control, *Aedes* removal), and week*treatment in the analyses of the effects of the *Aedes* removal treatment on weeks 1–7 [Online Resource 1] were no different from those in the analyses for weeks 4–7 with *T. rutilus* included as a covariate. Therefore, we excluded analyses of weeks 1–3 and focused on analyses testing for effects of both treatment and *T. rutilus* for weeks 4–7.

To test for the effects of *Aedes* removal on *Culex* development, we conducted a repeated-measures mixed model analysis (also Poisson distribution) on the combined number of *Culex* 4th instars and pupae (hereafter: mature *Culex*). A decrease in the number of mature *Culex*, independent of total abundance, can indicate that *Culex* growth and development are being negatively affected by *Aedes* competition or by *T. rutilus* predation. The former would provide evidence for competitive suppression of *Culex* by *Aedes*, whereas the latter would provide evidence that *T. rutilus* is reducing the longevity of *Culex* larvae, rendering them less likely to reach maturity. We also conducted a mixed model analysis on the combined numbers of *Culex* 1st and 2nd instars (hereafter: immature *Culex*) as an indirect test of oviposition behavior. Larger numbers of young instars in the *Aedes* removal containers could indicate greater oviposition by *Culex* in these containers.

To test for the effects of *Aedes* removal on predator colonization and abundance, we conducted two different analyses. We analyzed the effect of treatment on time to first colonization of *T. rutilus* for weeks 1–7 using survival analysis with a Weibull distribution

(PROC LIFEREG, SAS 9.1, SAS Institute 1990). This approach treats *T. rutilus* colonization as the focal event and compares treatments, testing whether the removal of *Aedes* delayed the arrival of the predator. We also tested the effect of treatment, as well as the random location effect, on *T. rutilus* abundance over time using a mixed model analysis with a Poisson distribution (PROC GLIMMIX, SAS 9.3).

For the in-lab bioassay, we conducted a one-way fixed effect ANOVA (PROC GLM, SAS 9.1, SAS Institute 1990) to test for differences in cumulative predation among all prey species. We then conducted orthogonal contrasts to compare vulnerabilities of the two genera, as well as to compare vulnerabilities of species within *Culex* and within *Aedes*.

Results

Effects of *Aedes* removal on *Aedes* and *Culex*

Species composition of all *Aedes* collected during the entire 7 weeks of the study included *A. japonicus* (93.5%), *A. triseriatus* (6.2%) and *A. albopictus* (0.3%). *Aedes* abundance was significantly and negatively affected by the *Aedes* removal treatment across all weeks (Table 1), with a reduction of over 90% between control and treatment containers. Small numbers of *Aedes* in the removal treatment could have resulted from eggs dropping off the aluminum sheeting into the container, or rainfall flooding eggs and causing hatching on days when containers were not checked. Nevertheless, mean ($\pm$ SE) *Aedes* abundance was 6.1 ± 0.8 larvae per sample in control containers, and 0.6 ± 0.1 larvae per sample in removal containers (Fig. 1) and remained consistently low over time [Online Resource 2]. The number of mature *Aedes* was also significantly reduced (Table 1, Fig. 1).

Culex composition of containers was *C. restuans* (82.9%), *C. pipiens* (9.4%), and *C. territans* (7.8%). Overall abundance of *Culex* was significantly greater in the *Aedes* removal treatment than in the control (Table 2, Fig. 1), with this difference in abundance appearing primarily during Week 7 of the experiment [Online Resource 2]. The number of mature *Culex* was also significantly greater in *Aedes* removal containers (Table 2, Fig. 1). The mean number of mature *Culex* was consistently greater in these containers across all weeks, significantly so in weeks 4 and 7 [Online Resource 3]. The number of immature *Culex* significantly decreased over time, but was not significantly affected by removal of *Aedes* (Table 2) [Online Resource 3]. There was a significant 3-way interaction of week, treatment, and *T. rutilus* abundance for both immature and mature *Culex* (Table 2). Immature *Culex* in the control treatment were significantly and positively correlated with *T. rutilus* abundance during weeks 4 and 6, and negatively correlated during week 7 [Online Resource 4], while in the *Aedes* removal treatment immature *Culex* were significantly and negatively correlated with *T. rutilus* only during week 5 [Online Resource 4]. Mature *Culex* were significantly and negatively correlated with *T. rutilus* in the control treatment during weeks 4, 5, and 6, and negatively correlated with *T. rutilus* in the *Aedes* removal treatment during weeks 6 and 7 [Online Resource 4].

Effects of *Aedes* removal on *T. rutilus*

Survival analysis yielded no significant effect of *Aedes* removal on colonization time of *T. rutilus* ($\chi^2 = 0.0406$, $p = 0.8404$, Fig. 3). There was a significant location effect on *T. rutilus* abundance, indicating that the 40 sites varied in their overall *T. rutilus* abundance. There was also a significant increase in *T. rutilus* colonization over time. However, *T. rutilus* abundance did not differ significantly between control and *Aedes* removal treatment containers (Table 3, Fig. 4).

Effects of *T. rutilus* on *Aedes* and *Culex*

Mixed model analyses showed a significant 3-way interaction between *T. rutilus* abundance, week, and treatment for both *Aedes* (Table 1) and *Culex* (Table 2). Effects of *T. rutilus* on *Aedes* and *Culex* were not significant during week 4 (Fig. 2A & E) and week 5 (Fig. 2B & F). However, during week 6 *T. rutilus* negatively affected *Aedes* and *Culex* in both treatments (Fig. 2C & G). During week 7, *T. rutilus* significantly and negatively affected only *Aedes* abundance in the control treatment, whereas only *Culex* was significantly affected in the *Aedes* removal treatment (Fig. 2D & H).

ANOVA of the in-lab bioassay showed significant differences among species in predator vulnerabilities (Table 4). An orthogonal contrast at the genus level showed that *Culex* was significantly more vulnerable than *Aedes* to predation by *T. rutilus* (Table 4, Fig. 5).

Discussion

Our first hypothesis, that early-colonizing *Culex* decline because of competition from later-colonizing *Aedes* (i.e., colonization/competition tradeoff) predicted that removal of *Aedes* from containers should increase the number of *Culex* or the number of mature *Culex* when compared to containers where *Aedes* are allowed to colonize. During the initial weeks analyzed, total *Culex* abundances were not significantly different between control vs. *Aedes* removal containers, but the number of mature *Culex* (4th instar larvae and pupae) was significantly greater in the *Aedes* removal containers. This suggests that *Culex* adults were continuing to oviposit in both containers, but *Culex* larvae in *Aedes* removal containers were maturing at a higher rate than *Culex* in the control containers. Total *Culex* abundances decreased in subsequent weeks in both types of containers (Fig. 2E, F, G, H) [Online Resource 2] and mature *Culex* abundance decreased and grew more variable [Online Resource 3] as *T. rutilus* numbers increased (Fig. 3). However, removal of *Aedes* attenuated the decrease in total *Culex* abundance over time relative to control, which resulted in greater number of *Culex* in *Aedes* removal containers by Week 7. These data, along with the significant negative effect of *T. rutilus* on *Culex* abundance, supports the hypothesis that *Culex* are negatively affected by both *T. rutilus* predation and *Aedes* competition. This result is thus consistent with the colonization/competition hypothesis for succession suggesting that the shift from *Culex* dominance early to *Aedes* dominance later is driven by competitive impacts of *Aedes* on *Culex*.

An alternative explanation for reduced *Culex* abundance where *Aedes* or *T. rutilus* are abundant may be oviposition avoidance behavior exhibited by adult *Culex* females, rather than direct consumption of *Culex* by *T. rutilus* or by competition from *Aedes*. *Culex* are well known to avoid ovipositing in containers with predators or predator cues (e.g., Van Dam and Walton 2008, Blaustein et al. 2004, Angelon and Petranka 2002), although it has not been specifically demonstrated that *Culex* avoids the predator *T. rutilus*. Similarly, *Culex* prefer to oviposit in nutrient-rich containers and avoid ovipositing in containers with high larval densities (Reiskind and Wilson 2004), although it has not been demonstrated that *Culex* specifically avoid ovipositing in containers with *Aedes*. It is clear that our *Aedes* removal treatment lowered total larval mosquito density, and may have resulted in increased nutrient availability, thus creating an environment more attractive to *Culex* oviposition.

Two pieces of evidence refute the hypothesis that *Culex* reduction is driven solely by lack of oviposition. First, immature *Culex* abundances did not significantly differ between treatments, whereas mature *Culex* abundances did. Were *Culex* exhibiting oviposition avoidance behavior toward *Aedes* we would expect to find fewer immature larvae in the control containers where *Aedes* abundances were greater. The disproportionate decrease in the number of mature vs. immature larvae instead suggests that *Culex* oviposition occurred

equally in both types of containers, after which their growth, development, or survival were suppressed by competition from *Aedes* that were more abundant in the control. Similarly, if *Culex* females avoided ovipositing where *T. rutilus* was abundant, we would expect numbers of early instar *Culex* to decline consistently with *T. rutilus* abundances. Though *T. rutilus* abundances had a three-way interaction effect on young *Culex* abundances, that effect was heterogeneous and not consistently negative, suggesting no strong pattern of avoiding oviposition where *T. rutilus* were abundant. Second, continued colonization and development of *T. rutilus* despite *Aedes* removal indicates that *T. rutilus* predation on *Culex* was likely high. *Toxorhynchites* larvae consume large numbers of prey (up to 400 prey per *T. rutilus* larva), and will readily cannibalize each other in the absence of prey (Jones and Schreiber 1994b). Prey density in these containers during Weeks 5–7 was suboptimal, with control buckets containing 211.71 ± 38.29 (mean $\pm$ SE) prey larvae per *T. rutilus* larva (per container), and *Aedes* removal buckets containing 110.68 ± 17.95 (mean $\pm$ SE) prey larvae per *T. rutilus* larva. Yet, despite these suboptimal prey abundances, *T. rutilus* abundance did not decrease over Weeks 5–7, and was also not affected by *Aedes* removal despite the lower number of prey larvae in the *Aedes* removed containers. *Culex* and *Aedes* comprised 93.5% of all potential prey larvae collected in the samples, so it is highly unlikely that *T. rutilus* was subsisting primarily on prey species other than *Aedes* or *Culex*. These collective results indicate that *Culex* were being consumed, probably at a relatively high rate, by *T. rutilus*.

Our second hypothesis, that *Culex* are less vulnerable to predation than *Aedes* (i.e., direct competition/predation tradeoff), predicted that removal of *Aedes* should cause a delay in the colonization of *T. rutilus* or a general decrease in *T. rutilus* abundance, due to their primary food source (*Aedes*) being eliminated and replaced by a taxon postulated to be less vulnerable to predation. In this study, neither *T. rutilus* overall abundance nor its colonization time were significantly affected by treatments. *Toxorhynchites rutilus* abundance significantly and negatively affected both *Aedes* and *Culex* abundances, presumably through the consumption of both genera. More directly, the in-lab bioassay showed that these *Culex* species were, if anything, significantly more vulnerable to predation than these *Aedes* species. Our data, in conjunction with previous studies that have consistently shown *Culex* to be inferior competitors to *Aedes* (Carrieri et al. 2003, Costanzo et al. 2005, Juliano 2009), suggest there is no direct competition/predation vulnerability tradeoff for these two genera. Results in this field experiment necessarily represent a combination of the effects of traits of the species within these genera that are common at our site, but for those species, the data collectively refute the hypothesis that *Culex* is less vulnerable to predation than the more competitive *Aedes*.

The greater vulnerability of *Culex* to predation by *T. rutilus* suggests another possibility: a direct tradeoff between colonization ability and predator vulnerability. Wellborn et al. (1996) proposed that a colonization-predation tradeoff may occur among species in ephemeral freshwater habitats. Specifically, species can avoid predation, and therefore do not require behavioral or morphological resistance to predation, by colonizing more ephemeral freshwater habitats where predators tend to be scarce (Wellborn et al. 1996). All three taxa in this study, including the predator, are adapted to relatively ephemeral habitats (e.g., treeholes and containers, Bradshaw and Holzapfel 1988, Juliano 2007) and although *Culex* tend to occupy slightly larger (and thus potentially more permanent) aquatic habitats than do *Aedes* (Rey et al. 2006, Yee et al. 2010) *Culex* seem to be specialized to be the earliest colonists of those habitats. We propose that the colonization-predation tradeoff among these mosquitoes depends primarily upon temporal, rather than spatial, separation from the predator, resulting from *Culex*'s ability to colonize containers earlier than *T. rutilus* and *Aedes*.

By Week 7, *T. rutilus* abundance had significant negative effects only on *Aedes* in the control containers, and only on *Culex* in the *Aedes* removal containers. It seems likely that *Culex* abundance in the control and *Aedes* abundance in the *Aedes* removal treatment were too low to detect effects of predation by *T. rutilus*. Alternatively, this may be evidence that *T. rutilus* exhibits prey switching behavior, preferentially attacking the prey type that is most abundant. Switching has been documented in other aquatic insect predators (Elliot 2004, Bergelson 1985) including other species of *Toxorhynchites* (Amalraj and Das 1996). *Toxorhynchites rutilus* has been observed to alter its behavior from capturing larvae to actively seeking and consuming *T. rutilus* eggs (Linley and Darling 1993) and terrestrial insects trapped on the surface of the water (Linley 1995). Switching may be another mechanism for prey species to avoid predation (i.e., by being rare) that does not rely on any direct behavioral, physical, or chemical adaptations of the larvae to predation.

If *T. rutilus* is able to consume *Culex* larvae, why does this predator not colonize containers sooner, when *Culex* are abundant? The delayed colonization of *T. rutilus* into containers could occur for at least two reasons. First, because *T. rutilus* overwinters as a larva (Bates 1949, Dodge 1964) and therefore is subject to freezing and desiccation during winter months, it may not be sufficiently abundant in early summer to colonize widely the containers in this study. This is likely not the full explanation for lack of *T. rutilus* colonization, because we have documented *T. rutilus* colonization in containers at this research site as early as June 4 (EG Murrell, unpublished data). Nevertheless, *T. rutilus* do appear to be less common during the early summer, so this factor may contribute to the lack of early *T. rutilus* colonization, which provides an opportunity for early-colonizing taxa such as *Culex* to develop in a predator-free environment. Second, *T. rutilus* oviposits preferentially in water from older treeholes that is infused with prey cues (Jones and Schreiber 1994a, Bradshaw and Holzapfel 1983). Because *T. rutilus* larvae have a relatively long development time and require sufficient prey to complete development, this preference is presumed to indicate that adult females show adaptive oviposition, preferring those containers that exhibit the chemical characteristics of a more permanent aquatic environment with ample prey (Bradshaw and Holzapfel 1983). Newer containers, despite their abundance of prey larvae and large amounts of water, may lack the chemical cues used by *T. rutilus* to identify them as appropriately permanent habitat for their offspring.

The results from this field manipulation, in conjunction with previous competition experiments, support the hypothesis that a colonization/competition tradeoff exists between *Culex* and *Aedes*. Though previous laboratory studies have demonstrated the competitive superiority of *Aedes* over *Culex* (Carrieri et al. 2003, Costanzo et al. 2005, Juliano 2009, Murrell and Juliano 2012), this is the first time *Aedes* suppression of *Culex* abundance has been explicitly demonstrated in the field by directly manipulating *Aedes* abundance, and the first time this tradeoff has been linked explicitly to succession of species in containers in the field. Because we manipulated only the abundance of *Aedes*, our field data cannot definitively show that *Aedes* are superior competitors to *Culex* (though this superiority is amply demonstrated in previous experiments - Carrieri et al. 2003, Costanzo et al. 2005, Murrell and Juliano 2012). Nevertheless, our results show that *Culex* abundance is negatively impacted by *Aedes* density in nature, and thus show that interspecific competition is an active important process under field conditions. Previous removal studies with plant systems (Pinder 1975), sessile marine invertebrates (Menge 1976), and rodents (Higgs and Fox 1993) have shown a similar improvement in performance of early colonizers when later colonizing, but competitively superior, species are removed. Our study shows that this same ecological pattern impacts early successional mosquito species. This result showing the importance of a colonization/competition tradeoff in this system suggests that this tradeoff may be an important mechanism of invertebrate succession in general.

Despite evidence of a colonization/competition tradeoff, we find no evidence of a competition/predation vulnerability tradeoff for these two genera. This suggests that the relationship of predator vulnerability of species in this community is more complicated than a simple tradeoff with competitive ability, and should instead be considered in the context of multiple life history characteristics. As a factor affecting ecological succession, the competition/predation tradeoff does not appear to predict the colonization order of the genera we studied. Our results also suggest that *T. rutilus* is capable of affecting species composition of container communities even in the absence of a direct competition/predation tradeoff among prey species. Collectively, our results highlight some of the species interactions that determine how succession proceeds in communities. Further research with these communities could help us to understand how multiple tradeoffs among species can influence change in community composition over time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Back-transformed least square mean ($\pm$ SE) larval abundances per sample (mean sample size 627.8 ± 3.4 mL) of all *Culex* mosquitoes, mature *Culex* only, all *Aedes* mosquitoes and only mature *Aedes* for control or *Aedes*-removed treatments in water-filled containers at the Tyson Research Center in Eureka, Missouri, USA in 2010. Treatment effect was highly significant for all four groups (mixed models, $p < 0.0001$)

Figure 2.

Larval abundances per sample (mean sample size 627.8 ± 3.4 mL) of *Aedes* (left column) and *Culex* (right column) in response to *T. rutilus* abundance per container for control and *Aedes* removal treatments in (a,b) Week 4, (c,d) Week 5, (e,f) Week 6 and (g,h) Week 7. Data are ln-transformed, with points on the x-axis representing samples which contained no larvae. The size of individual data points corresponds with the number of individual samples that had identical values. Lines represent the relationships of prey larval densities to *T. rutilus* abundance as predicted by the mixed model analyses

Figure 3.
Effects of *Aedes* removal treatment on *T. rutilus* time to initial colonization

Figure 4. Abundance of *T. rutilus* per container (mean $\pm$ SE) over time in the control vs. *Aedes* removal treatment

Figure 5. Least square mean ($\pm$ SE) cumulative prey consumed of each species and genus by *T. rutilus* in the bioassay

Mixed model results for effects of treatment, week, *T. rutilus* abundance, and all interactions on the total larval abundances and abundances of mature individuals for *Aedes*. Significant effects are in **bold print**.

Table 1

Parameter	Total <i>Aedes</i> Abundance			Mature <i>Aedes</i> Abundance		
	df	F Value	P value	df	F Value	P value
Week	3,156	3.99	0.0090	3,156	3.00	0.0324
Treatment	1,148	465.28	<0.0001	1,151	114.08	<0.0001
Week*Treatment	3,148	4.08	0.0081	3,151	0.18	0.9085
<i>T. rutilus</i>	1,148	5.46	0.0208	1,151	6.45	0.0121
<i>T. rutilus</i> *Week	3,148	1.28	0.2839	3,151	6.90	0.0002
<i>T. rutilus</i> *Treatment	1,148	0.02	0.8935	1,151	0.20	0.6529
<i>T. rutilus</i> *Week*Treatment	3,148	2.90	0.0371	—	—	—

Mixed model results for effects of treatment, week, *T. rutilus* abundance, and all interactions on the total larval abundances and abundances of immature and mature individuals for *Culex*. Significant effects are in **bold print**.

Table 2

Parameter	Total <i>Culex</i> Abundance			Immature <i>Culex</i> Abundance			Mature <i>Culex</i> Abundance		
	df	F Value	P value	Df	F Value	P value	df	F Value	P value
Week	3,156	83.44	<0.0001	3,156	31.47	<0.0001	3,156	56.31	<0.0001
Treatment	1,148	21.58	<0.0001	1,148	1.29	0.2586	1,148	36.91	<0.0001
Week*Treatment	3,148	5.85	0.0008	3,148	2.26	0.0834	3,148	8.87	<0.0001
<i>T. rutilus</i>	1,148	3.16	0.0775	1,148	0.96	0.3284	1,148	29.13	<0.0001
<i>T. rutilus</i> *Week	3,148	10.23	<0.0001	3,148	7.38	0.0001	3,148	5.31	0.0017
<i>T. rutilus</i> *Treatment	1,148	13.51	0.0003	1,148	2.53	0.1137	1,148	2.32	0.1303
<i>T. rutilus</i> *Week*Treatment	3,148	3.65	0.0140	3,148	8.28	<0.0001	3,148	8.34	<0.0001

Table 3

Mixed model results for effects of location, time, and treatment on *T. rutilus* abundance. Significant effects are in **bold print**.

Parameter	Estimate	df	F Value	P value
Location	0.7912			
Week		3,156	13.9	<0.0001
Treatment		1,156	0.26	0.6096
Week*Treatment		3,156	1.26	0.2904

Table 4

ANOVA results for species and genus differences in cumulative prey eaten by *T. rutilus* in the in-lab bioassay. Significant effects and comparisons are in **bold print**.

Source	Comparison	df	F value	P value
Species		3	3.94	0.0225
	<i>C. pipiens</i> vs <i>C. restuans</i>	1	4.09	0.0561
	<i>A. triseriatus</i> vs. <i>A. japonicus</i>	1	0.06	0.8032
	<i>Culex</i> vs. <i>Aedes</i>	1	8.47	0.0084
Error		21		