Larval Competition Between Aedes and Culex sp. (Diptera: Culicidae) found in Texas on Artificial Diets

Everett Alford, Luis Flores, Haley Gavranovic, Kristal Lozano, Bethany Mikles, Mikayla Monk, Isaac Neuman, Rachel Sexton, Dr. Gabriel Hamer

Texas A&M University, Department of Entomology

Editor: Dawson Tidwell

Abstract: Mosquitoes (Diptera: Culicidae) are notorious vectors of diseases that pose serious threats to human health, making them highly relevant topics of study within many research laboratories. Studies to further understand mosquito biology and ecology are vital to develop more cohesive epidemiological models and effective methods of vector control, making the need for a fuller understanding of interspecies larval competition and its effect on mosquito fitness particularly important. In this experiment, the ability of resource procurement of *Aedes aegypti* and *Culex quinquefasciatus* larvae was tested against interspecies competition in a laboratory setting in an attempt to determine which species would show an increased fecundity specifically in a lab setting. Eggs of each species were reared till adulthood and their numbers of emergence were recorded in order to observe the effects of interspecies competition in the larval stage on fitness. Results collected concerning larval mortality showed no obvious advantage for either species, showing similar rates of survival despite competition for food resources. These results suggest that the two species are relatively equally matched at the larval stage, but further refined experiments and analysis are required to ascertain the full effects of competition between these species.

Keywords: Diptera, Culicidae, interspecies competition, *Aedes aegypti*, *Culex quinquefasciatus*, larval competition

Mosquitoes (Diptera: Culicidae) are among the most medically influential groups of insects, acting as highly efficient vectors of malaria, lymphatic filariasis, and various other viral infections that infect nearly 500 million people every year (Beerntsen 2000). Many attempts have been made to study and control the transmission and spread of these widespread diseases with various appr-oaches ranging from the use of biological control, mass drug administration and distribution of personal protective devices, to the combination of methodologies within integrated vector control plans (Merritt et al. 1992, Reiskind and Lounibos 2009). As mosquitoes are holometabolous, undergoing an egg, larval, pupal, and adult stage, a great deal of study has been conducted on its immature aquatic stages. These stages are typically those that are found near and

around human dwellings within man-made containers such as old rubber tires, buckets, or cemetery vases (Juliano 1998, Yee and Juliano 2004).

To study the larval stage, laboratory-reared mosquito colonies are often maintained for use in experiments, though upkeep of these colonies can often be labor-intensive and time-consuming, requiring meticulous record keeping and monitoring of appropriate conditions for each developmental stage (Kauffman et al. 2017). Many methods have been formulated in detail in order to regulate and successfully rear mosquito colonies. These methods are often different depending on species, as egg-laying habits and developmental intervals vary for each. For example, Culex spp. are known to lay egg rafts of about 250-300 eggs directly onto water surfaces, which will hatch into larvae within 48 hours. Aedes spp, on the other hand, lay eggs individually onto damp substrates above the water line, typically filter paper or a coffee filter, and will hatch anywhere from within a few days time to several months later (Kauffman et al 2017, Imam et al. 2014).

While highly sensitive and delicate screening methods, sophisticated costly diets, or strictly monitored laboratories may be required for the rearing of some strains, many laboratories do not possess these capabilities, and so methods that may be performed in any laboratory are preferable (Imam et al. 2014). For this reason, once hatched, the larvae are often fed readily-available, cheap, and preservable diets such as brewers yeast, cat food, bovine liver powder, or tetramin fish food (Imam et al. 2014, Pugglioli et al. 2013). This phase of rearing plays a vital part in the mosquito developmental cycle which will have lasting effects on the continued survival and adult emergence with many factors such as larval diet quality, food scarcity, and larval competition for food. Studies have shown that each of these components directly and often irreversibly affect adult fitness and beyond; competition among larvae is a supported hypothesis for patterns of invasion and decline of species, and food availability has a positive relationship with larvae and pupae numbers within laboratory colonies (Pugliolli et al. 2013, Juliano 1998, Aznar et al. 2018).

These larvae are the key to the proper establishment of epidemiological models of vector-borne disease transmission and of vector population regulation. The true extent of ecological principles and the effects of competition for resources in larval mosquitoes on adult longevity has yet to be fully understood and requires further study. (Reskind and Lounibos 2009). This experiment attempted to compile further data about these relationships by examining the competition between Aedes aegypti (L.) and Culex quinquefasciatus (Say 1823) larvae for a commonly used artificial diet, and the effects of this competition on each species' development into the adult stage.

Materials and Methods

F1 *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes were caught in the wild in the San Antonio area (SATX) and the Bryan-College Station area (BCS), respectively, and maintained within a lab colony within the Hamer Laboratory at

Texas A&M University. The F5 eggs of both species were used within the experiment.

Two trials were performed simultaneously. 50 eggs of each species were added to two larval trays (BioQuip, Rancho Dominquez, CA) containing 500 mL of distilled water and 2000 µL of aqueous liver powder (MP Biomedicals, Santa Ana, CA), and will be henceforth referred to as Trial 1 and Trial 2. Two additional control larval trays were also similarly prepared containing only a single species each. Temperature and humidity were kept constant at 80℉ for all trays.

Eggs were allowed to develop and were observed for two weeks. Observations were taken on the emergence and survivorship rates among both species during the two-week process. After the eggs hatched into larvae, the number of survivors, the amount of larvae that transformed into pupae, and the number of adults that emerged were recorded. Once all remaining pupae emerged into adults, the specimens were transferred to bug dorm containers (BioQuip Inc., CA, USA). They were then frozen and classified into their species under a light microscope. All data was recorded and inserted into tables for analysis.

Results

In the control trays of both trials, both species had a survival rate of 100 percent. For the trays with the food source, both species showed relatively similar survival rates. In Trial 1, 45 Culex and 48 Aedes larvae survived to adulthood, resulting in a 90 percent Culex survival rate and a 96 percent Aedes survival rate. In Trial 2, 47 Culex and 42 Aedes larvae survived to adulthood, resulting in a 94 percent Culex survival rate and a 84 percent Aedes survival rate. Aedes adults showed higher rates of survival in Trial 1, while Culex showed higher rates of survival in Trial 2, which can be seen in Table 1.

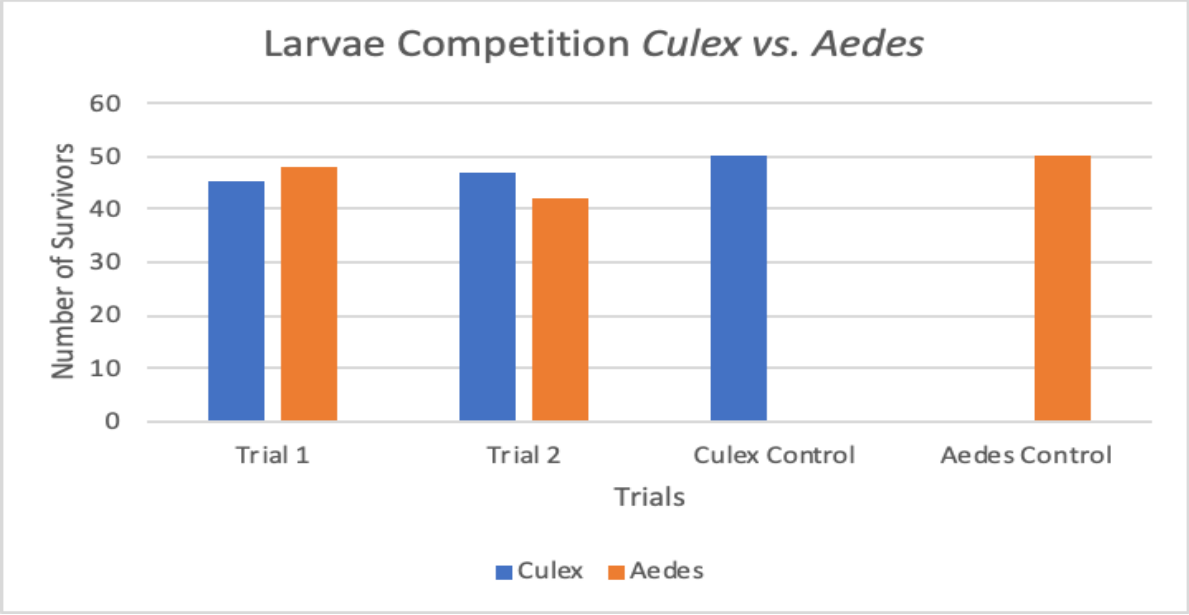

Table 1: The results of Trials 1 and 2, showing the number of eggs that survived to adulthood for each species. *Culex* are shown in blue and *Aedes* in orange. The controls show equal survival for each species.

Discussion

Results suggest that there was equal competition between the two species of mosquitoes. Near equal competition when Trials 1 and 2 are compared, as each trial showed one species surviving more than the other. The *Aedes* out performed the *Culex* by a small margin of six percent in the first trial, whereas in the second trial *Culex* survived over *Aedes* by 8 percent. Along with the 100 percent survival rate for both species in the controls, these minimal differences show that there was essentially even competition between the two species. Both *Culex* and *Aedes* were shown to be capable of competing with each other for food resources.

The results of this experiment and the lack of significant differences in survival between species may be due to the experimental design. Only two trials were prepared in addition to the control, which may have somewhat limited the true extent to which competition between the species may have been observed. A larger sample size, perhaps with 5 or 6 trays of each species, may produce different results.

Further study of larval interspecies and intraspecies competition may also further explain the results of this experiment, as interactions and competition for resources may differ between mosquitoes of the same species as compared to competition between different species. This information might provide valuable insight into the relationship of larvae species with resource acquisition and habitat viability.

The similarities in survival rate between these species might have something to do with the geographic distribution of these mosquitoes as well. Both species were recovered within Texas and maintained within laboratory colonies, so their adaptation to competition with each other in the wild may explain their success in competing with each other in a laboratory environment. These species occupy much of the same environment, so this equality in competition for food resources even under lab conditions might be the result of evolutionary pressures that resulted in convergence of these traits. This study has led to more questions for future research regarding the competitiveness of these two species related to the advantages they develop in their environments.

References

Aznar, V. R., I. Alem, M. S. D. Majo, B. Byttebier, H. G. Solari, and S. Fischer. 2018. Effects of scarcity and excess of larval food on life history traits ofAedes aegypti(Diptera: Culicidae). J. Vector Ecol. 43: 117–124.

Beerntsen, B. T., A. A. James, and B. M. Christensen. 2000. Genetics of Mosquito Vector Competence. Microbiology and Molecular Biology Reviews. 64: 115–137.

Bond, J. G., A. Ramírez-Osorio, C. F. Marina, I. Fernández-Salas, P. Liedo, A. Dor, and T. Williams. 2017. Efficiency of two larval diets for mass-rearing of the mosquito Aedes aegypti. Plos One. 12.

Imam, H., G. Sofi, Zarnigar, and S. Aziz. 2014. The basic rules and methods of mosquito rearing (Aedes aegypti). Trop. Parasitol. 4: 53.

Juliano, S. A. 1998. Species Introduction And Replacement Among Mosquitoes: Interspecific Resource Competition Or Apparent Competition? Ecology. 79: 255–268.

Kauffman, E., A. Payne, M. Franke, M. Schmid, E. Harris, and L. Kramer. 2017. Rearing of Culex spp. and Aedes spp. Mosquitoes. Bio Protoc. 7.

Puggioli, A., F. Balestrino, D. Damiens, R. S. Lees, S. M. Soliban, O. Madakacherry, M. L. Dindo, R. Bellini, and J. Gilles. 2013. Efficiency of Three Diets for Larval Development in Mass Rearing Aedes albopictus (Diptera: Culicidae). J. Med.Entomol. 50: 819–825.

Reiskind, M. H., and L. P. Lounibos. 2009. Effects of intraspecific larval competition on adult longevity in the mosquitoesAedes aegyptiandAedes albopictus. Med. Vet. Entomol. 23: 62–68.

Souza, R. S., F. Virginio, T. I. S. Riback, L. Suesdek, J. B. Barufi, and F. A. Genta. 2019. Microorganism-Based Larval Diets Affect Mosquito Development, Size and Nutritional Reserves in the Yellow Fever Mosquito Aedes aegypti (Diptera: Culicidae). Front. Physiol. 10.

Yee, D. A., B. Kesavaraju, and S. A. Juliano. 2004. Larval feeding behavior of three co-occurring species of container mosquitoes. J. Vector Ecol. 29: 315–322.