# Effects of Larval Diet on Life History Traits of *Culex* Mosquitoes

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ABSTRACT: The responses of life history characters to changes in abundance of larval diet were examined for *Culex quinquefasciatus*, *Culex stigmatosoma* and *Culex tarsalis*. Differences in larval habitat preferences were reflected in laboratory studies that evaluated development and survivorship across ten food concentration treatments. Survivorship for *C. tarsalis* larvae rapidly declined at high food concentrations. Immature developmental times of *C. quinquefasciatus* and *C. stigmatosoma*, species commonly found in hypereutrophic waters, were significantly shorter than for *C. tarsalis*. Despite similar larval habitat preferences, *C. quinquefasciatus* and *C. stigmatosoma* had comparatively small and large adult body sizes, respectively. *Culex tarsalis* adults were noticeably larger and heavier than *C. quinquefasciatus* and, occasionally, smaller and lighter than *C. stigmatosoma*.

## INTRODUCTION

Mosquitoes serve as vectors of arboviruses and filarial worms and transmit these pathogens and parasites to other animals, resulting in diseases such as avian malaria, human filariases, encephalitis and West Nile Virus (Goddard 2003). To reduce the threat to human health and level of nuisance, the life cycle and potential mosquito production from different habitat types must be well understood. Food levels for mosquito larvae often differ appreciably among potential developmental sites, resulting in differences in survivorship and adult size. Three *Culex* species are commonly found in California: *Culex quinquefasciatus*, *Culex tarsalis* and *Culex stigmatosoma* (Bohart and Washino 1978). Larval habitats of *C. quinquefasciatus* generally include artificial containers, water barrels, basins and ground pools with minimal plant shelter (Laird 1988). *Culex tarsalis* is generally found in water associated with agricultural usages, and *C. tarsalis* larvae are less tolerant of water containing high concentrations of suspended matter than are *C. stigmatosoma* and *C. quinquefasciatus* larvae (Reisen et al. 1992). *Culex stigmatosoma* larvae are generally found in highly enriched waters such as sewage drains, cesspools, secondary treatment sewage ponds and dairy pastures, but are capable of living in waters with low concentrations of suspended matter.

The objective of this study was to examine the responses of three life history traits of the three *Culex* species across a range of food resource levels that are characteristic of larval mosquito developmental sites in nature. The effects of food availability on larval survivorship, developmental time and body size (wing length and mass) at eclosion were examined. Food levels were chosen to represent a range of food quantity across aquatic environments where larvae of the three species are naturally found, ranging from the comparatively low food availability characteristic of wetlands to the high food levels characteristic of ponds containing large amounts of organic matter.

## METHODS AND MATERIALS

*Culex stigmatosoma* and *C. tarsalis* colonies were established from egg rafts collected from the UCR Aquatic Research Facility (Riverside, CA) in 2006. *Culex quinquefasciatus* used in these experiments were from a laboratory colony (BtSyn strain) derived from several populations in southern California in 1990 (Georghiou and Wirth 1997). The larval diet consisted of three parts mouse chow to one part Brewer's yeast. Ten food treatment levels ranged from 25 µl to 2000 µl of a stock solution of 1.5 mg of larval diet per 50 µl administered every other day. Within 24 h of hatching, larvae were placed individually in 10 ml of deionized water and maintained in a temperature-controlled environment (23 ± 1 °C) with a 16:8 hr L:D cycle. Larval rearing containers were scanned, at minimum, thrice daily for water level maintenance, death, pupation and emergence. Sample size consisted of 30 larvae for each treatment per species per gender (repeated 3-10x). *Culex quinquefasciatus* were reared at only three food levels. After a successful emergence, each adult specimen was anesthetized and frozen (-20 °C) until further analysis. Each adult mosquito was dried overnight at 43 °C, weighed to the nearest microgram three times using a Sartorius M2P electronic microbalance, and a mean mass was calculated. Wing length was measured from the distal end of the alula to the end of the wing (along the R3 vein), minus the fringes at 10x using a stereodissecting microscope.

We analyzed life history data (by sex) using multiple analysis of variance (MANOVA; SAS Institute 1999) with wing length, dry mass and developmental time as dependent variables, and species, food concentration and interaction as the independent variables. Standardized canonical coefficients were used to interpret the contribution of each dependent variable to any significant effects. When significant effects were found, multiple post-hoc comparisons were carried out using Tukey's HSD. All results are given as mean ± 1 SE.

## RESULTS

Immature developmental time and body size (wing length and adult dry mass) of the three mosquito species did not change similarly across the gradient of food concentration examined in this study; the species by food concentration interaction was significant for each of the three *Culex* life history characters (♂: $F_{54,1776}$ = 6.10, p < 0.0001; ♀: $F_{54,1614}$ = 4.87, p < 0.0001). The standardized canonical coefficients for the species by food concentration interaction effect for males indicated that dry mass contributed most to species-specific differences among food levels on axis 1 and interspecific differences of developmental time across food levels contributed highly to axis 2 (Figure 1). For females, interspecific differences of body size were

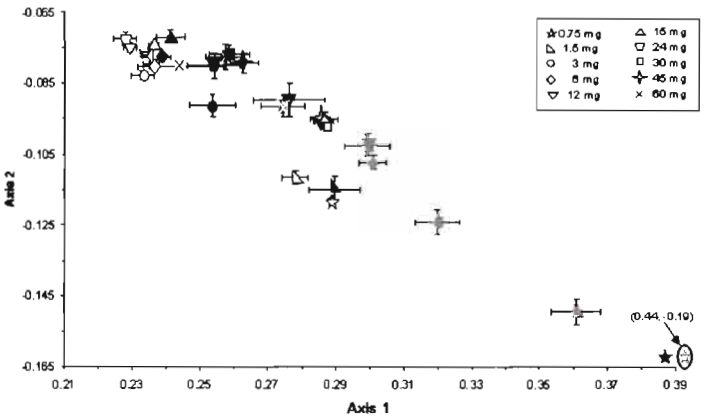


**Figure 1.** Bivariate means (± 1 SE) for standardized canonical coefficients for life history traits of males of three *Culex* species reared at ten food concentrations (0.75 – 60.00 mg/d). White, black and gray symbols represent *C. quinquefasciatus*, *C. stigmatosoma* and *C . tarsalis*, respectively. The response of *C. tarsalis* to the lowest food level (point surrounded by a circle) is off the scale illustrated.

important determinants of the significant interaction effect across food levels. Species-specific differences of wing length across food concentrations contributed the most to discriminant axis 1 and dry mass contributed most to interspecific differences of females responding across food levels along axis 2 (Figure 2). Increasing food concentration had a comparatively greater direct effect on adult dry mass than on the other life history characters. Regardless of food concentration, *C. tarsalis* required more time to complete immature development than did either *C. quinquefasciatus* or *C. stigmatosoma*. Wing length also contributed significantly to the differences among species in both sexes.

Survivorship to eclosion differed significantly between *C. stigmatosoma and C. tarsalis* and food concentrations (species: $F_{1,77}$ = 69.56, p < 0.0001; food concentration: $F_{9,77}$ = 10.68, p < 0.0001).

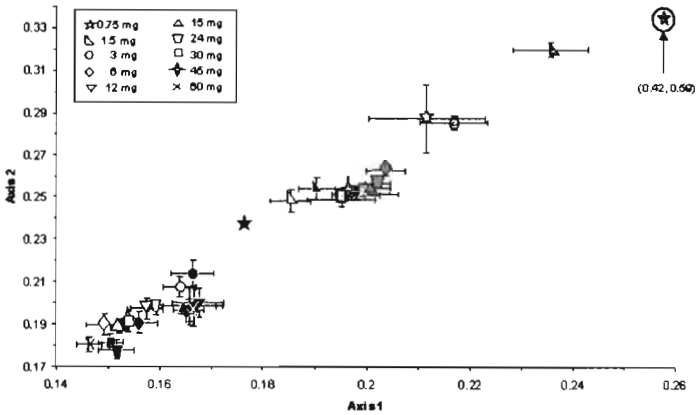


**Figure 2.** Bivariate means (± 1 SE) for standardized canonical coefficients for life history traits of females of three *Culex* species reared at ten food concentrations (0.75 – 60.00 mg/d). White, black and gray symbols represent *C. quinquefasciatus*, *C. stigmatosoma* and *C . tarsalis*, respectively. The response of *C. tarsalis* to the lowest food level (point surrounded by a circle) is off the scale illustrated.

The interaction of the two factors was not statistically significant ($F_{9,77}$ = 1.29, p = 0.257). Survivorship to eclosion of *C. tarsalis* increased directly within the lower food concentrations (0.75 – 3 mg/d), averaged > 60% at intermediate food concentrations (6 – 18 mg/d) and then declined appreciably at food levels > 18 mg/d . Survivorship to eclosion of *C. stigmatosoma* also increased directly across the three lowest food levels, but unlike *C. tarsalis* did not decline markedly at the four highest food levels tested. Across food concentration, *C. tarsalis* survivorship (38.11 ± 2.38%) was significantly less than *C. stigmatosoma* survivorship (73.94 ± 3.58%, p < 0.0001). For the three food treatment levels (1.5, 6 and 24 mg/d) where all species were reared, there was a significant difference for survivorship across species ($F_{2,35}$ = 10.88, p = 0.0002). *Culex tarsalis* survivorship (48.56 ± 4.17%) was significantly lower than both *C. quinquefasciatus* (81.11 ± 7.05%, p = 0.001) and *C. stigmatosoma* (76.30 ± 7.05%, p < 0.005). Survivorship significantly differed across species at medium and high food levels (Tukey tests, p < 0.05). Survival of *C. quinquefasciatus* and *C. stigmatosoma* was similar at each of the three food levels examined (Tukey tests, p > 0.05).

There were significant species, concentration and species by concentration effects for wing length, adult dry mass and developmental time (all p < 0.0001). Wing lengths of *C. stigmatosoma* adults were significantly longer (♂/♀: p < 0.0001) than the other two species. The same pattern was observed for adult dry mass. Individuals reared at low food concentrations (< 3 mg/d) were significantly smaller (♂/♀: Tukey tests, p < 0.0001) and less massive (♂/ ♀: Tukey tests, p < 0.01) than individuals at food concentrations > 3 mg/d. At the low food concentrations, differences across species were marginally significant (p≤ 0.05). As food resources became more readily available, differences

across species became more significant as maximum male wing lengths [2.90 ± 0.03 mm (24 mg/d), 3.59 ± 0.09 mm (18 mg/d), 3.08 ± 0.04 mm (12 mg/d)] and maximum male dry mass [0.549 ± 0.010 mg (24 mg/d), 0.713 ± 0.019 mg (12 mg/d) and 0.610 ± 0.011 mg (18 mg/d)] were obtained for *C. quinquefasciatus*, *C. stigmatosoma* and *C. tarsalis*, respectively. Females exhibited a similar pattern but were significantly larger and more massive than their male counterparts for a given food concentration (all $p < 0.05$) except for the lowest food concentration.

Unlike wing length and adult dry mass, developmental time significantly decreased with increasing food concentration. Across the gradient of food concentration examined here, immature developmental times for *C. tarsalis* in nearly all food treatments were significantly longer than for *C. quinquefasciatus* and *C. stigmatosoma* (♂/♀: Tukey tests, $p < 0.0001$).

## DISCUSSION

The comparatively lower survival of *C. tarsalis* larvae in the high food environments at the upper end of the food gradient indicates that poor larval survival is an important factor limiting its occurrence in the hypereutrophic developmental sites used by *C. stigmatosoma* and *C. quinquefasciatus*. Moreover, the low food conditions in these experiments did not seem to favor *C. tarsalis* larvae relative to the other species. *Culex tarsalis* immature development time was also longer at a particular food concentration than for the other species, and surprisingly low food levels characteristic of its larval developmental sites (e.g., wetlands, sumps in irrigated pastures) increased the developmental times of *C. tarsalis* more than for the other species. Because water in the rearing vessels was not changed on a daily basis, waste products presumably increased as larval diet increased. It is possible that *C. tarsalis* larvae are less capable of tolerating waste product buildup than are the larvae of the two species common in highly enriched habitats in nature. This might explain the severe reduction of survivorship at high food concentrations and the comparatively lower survivorship and longer developmental times of *C. tarsalis* larvae at all food concentrations.

As expected, females of each three *Culex* species were noticeably larger and heavier than their male counterparts with one exception; at the lowest food concentration, individuals had a mean dry mass of 0.20 mg, regardless of sex or species. As food became more available, the differences between the sexes became more apparent. The differences of body size at the low vs. high food levels were caused in part by a requirement for the minimum amount of larval nutritional resources necessary for basic metabolic processes and successful development. The second lowest food concentration administered to the larvae represents an important transition point for the three *Culex* species. Above this food concentration, survivorship approximates 50%, and species differences became more apparent with *C. stigmatosoma* adults growing significantly larger than either *C. tarsalis* or *C. quinquefasciatus* adults.

In contrast to body size, developmental time decreased with increasing food availability. Prolonging immature development, especially at the lower food concentrations, allowed for the accumulation of necessary resources for successful adult emergence, especially for females who require additional reserve for egg production. Because *C. tarsalis* larvae are generally found in low nutrient waters that can vary spatially and temporally (Walton et al. 1990), it is possible to encounter unsuitable habitats from time to time, selecting for a slower growth rate in this species. It is also possible that the observed patterns in developmental time (as well as survivorship) for *C. tarsalis* could be explained by species differences in larval feeding rate and particle size intake of *C. tarsalis* compared to either *C. quinquefasciatus* or *C. stigmatosoma*.

In summary, the observed patterns for life history traits indicate that nutrient resources were critical during larval development for these three *Culex* species at the lowest food treatment levels. Future research will focus on this narrower range, taking into account how differences in feeding rate, particle size intake and nutrient quality may impact the life history traits of *Culex* mosquitoes.

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