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Bionomics and Vector Potential of *Culex thriambus* (Diptera: Culicidae) Mosquitoes in Lake County, California

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Abstract

California statewide West Nile virus (WNV) minimum infection rates in *Culex thriambus* Dyar mosquitoes are high; however, few specimens are submitted and tested each year, as their distribution seems limited to larval habitats along riparian systems. To evaluate the role of *Cx. thriambus* in the amplification, maintenance, and overwintering of WNV in Lake County, CA, the bionomics and vector potential of the species was investigated during 2014 and 2015. *Culex thriambus* was the most abundant mosquito species, with 1,153 adults and 7,624 immatures collected by vacuum aspiration and dip sampling, respectively, at the primary study site. Detection of WNV in four mosquito pools during September through November coincided with peak seasonality. Females entered and maintained a reproductive diapause during winter under field and seminatural conditions. Diapause was initiated in the majority of *Cx. thriambus* females by October and was terminated by 30 March. Some parous females (7.1%) and those in host-seeking arrest (7.1%) were collected throughout the winter period. An accrual of 679.51 degree-days (°D) was necessary for diapause termination under seminatural conditions. *Culex thriambus* females fed on 16 different avian species during spring and summer, and no mammalian feeds were detected. West Nile viral RNA was detected in four of 42 *Cx. thriambus* pools tested during June through November and infection rates ranged from 3.53–28.15/1,000 tested. In summary, WNV transmission may be increased along riparian corridors throughout California where *Cx. thriambus* mosquitoes remain relatively abundant.

Key words: bionomics, Culex thriambus, West Nile virus, diapause, California

West Nile virus (WNV, *Flaviviridae: Flavivirus*) invaded California in 2003 and then overwintered successfully and amplified to epidemic levels during 2004 (Reisen et al. 2006b). The virus is maintained in an enzootic transmission cycle between *Culex* mosquitoes and passerine birds (Kramer and Bernard 2001). Specifically, *Culex tarsalis* Coquillett, members of the *Culex pipiens* complex, and *Culex stigmatosoma* Dyar are competent and efficient laboratory vectors of WNV (Goddard et al. 2002, Reisen et al. 2005) and are repeatedly implicated in the maintenance and amplification of the virus in California (Turell et al. 2005, Reisen et al. 2006c). Mosquito control and surveillance activities are focused on these key *Culex* species; however, comparable studies on other *Culex* vectors such as *Culex thriambus* Dyar are lacking. Although *Cx. thriambus* is a competent laboratory vector of WNV and has been found repeatedly infected in nature (Reisen 2006a), no study has thoroughly evaluated its biology and vector potential. Considered a relatively rare, riparian species in California (Reisen et al. 2006a), consequently its role in the transmission of WNV may be underappreciated, as adults are infrequently collected. According to Darsie and Ward (2005), *Cx. thriambus* may be found in much of California. Their range is limited to the east by the crest of the Sierra Nevada Mountains and by the deserts of Southern California. The northern limit of their range in the Central Valley is the Trinity Mountains; in the coastal mountains it includes the southern half of the Eel River watershed.

Lake County is located in Northern California between the Central Valley and the Pacific Coast and consists of mountainous terrain and agricultural land interspersed with rural communities. Several *Culex* vectors of WNV are present in Lake County, and WNV was first detected in the county in 2004 (Ryan et al. 2013). Immature and adult *Cx. tarsalis* and *Cx. stigmatosoma* are commonly collected throughout Lake County, while members of the *Cx. pipiens* complex are relatively uncommon (Breuner et al. 2013, Ryan et al. 2013). Adult *Cx. thriambus* are sporadically collected throughout Lake County (< 5 females per trap night) in carbon dioxide-baited (CO_2) traps. Recently, a large population of larval (up to 287 per dip) and adult (up to 109 adults per vacuum collection) *Cx. thriambus* were detected in Lake County. The present

study reports results of field and seminatural studies on aspects of the bionomics and vector potential for *Cx. thriambus* mosquitoes in Lake County, including relative dominance and seasonality, overwintering and mating, host selection, and WNV infection rates.

Materials and Methods

Study Area

The primary study area was located in Highland Springs Park and Recreation Area (38.9471 N, −122.9040 W) ~8 km southwest of Kelseyville, CA, at an elevation of 452 m. The Highland Springs Dam is an earthen dam, constructed in 1965, with an underground primary spillway that limits outflow from Highland Springs Reservoir (32.12-km^2 watershed) to Highland Creek. The primary spillway is 85.0 by 1.2 by 1.2 m (L by W by H) and adult mosquitoes, including *Cx. thriambus*, rest inside the spillway when outflow from the reservoir is reduced to <1.5 cubic meters per second (cms) during the summer and fall. Additionally, mosquito larvae were found in the stagnating water below the spillway that connects with Highland Creek.

Adult Collections

Mosquitoes were collected resting inside the primary spillway of Highland Springs Dam using a hand-held aspirator (modified Ryobi 18 Volt One+ Hand Vac, One World Technologies, Inc., Anderson, SC) on a weekly basis from January 2014 through December 2015, unless outflows from Highland Springs Reservoir prevented sampling. Additionally, two CO_2 traps were set out from 28 August through 6 November 2015 to augment numbers of *Cx. thriambus* for WN viral RNA detection in mosquito pools.

Resting collections were made during daylight hours between 8 a.m. and 10 a.m. and CO_2 traps were operated overnight and retrieved the following morning concurrent with resting collections. Following collection, mosquitoes were immediately transported to the laboratory where they were anesthetized and enumerated by species. *Culex* mosquitoes in the subgenus *Culex* were grouped into pools of ≤50 females per species, per collection method, frozen at −80 °C, and shipped on dry ice to the Davis Arbovirus Research and Training (DART) Laboratory at the University of California, Davis, for WN viral RNA testing by qRT-PCR (Brault et al. 2015). Up to 15 *Cx. thriambus* females collected weekly during October through April were dissected to determine diapause status before being pooled. Blood-fed *Cx. thriambus* mosquitoes were stored at −80 °C until used for bloodmeal analysis.

Larval Collections

The aquatic habitat created by outflow from the primary spillway of Highland Springs Dam was sampled for mosquito larvae on a weekly basis with a standard 350-ml polypropylene mosquito dipper (BioQuip Products, Inc., Rancho Dominguez, CA). Five dip collections were taken from Highland Creek at 2-m intervals, beginning at the spillway and moving downstream. Harborage for mosquito larvae was provided by fibrous root balls of Pacific willows (*Salix lucida*) lining the margins of the creek. All larvae and pupae were concentrated into a single sample, immediately taken to the laboratory, and enumerated to species and immature stage. Pupae were placed in emergence containers and identified as adults. Larvae and adults were identified according to Darsie and Ward (2005) and Meyer and Durso (1988). Samples were collected beginning in January 2015 and continued through December 2015.

Overwintering Studies

Outflows from Highland Springs Dam limited collections of adult *Cx. thriambus* during winter, so overwintering studies in a seminatural outdoor enclosure ("mosquito house"; Nelms et al. 2013) were conducted. To delineate diapause induction and termination cues utilized by *Cx. thriambus* mosquitoes of known age and emergence site, a mosquito house was used to house overwintering mosquitoes during the winter (September–March) of 2015–2016. The mosquito house was located at the Lake County Vector Control District (LCVCD) in Lakeport, CA (39.0391 N, −122.9149 W) and positioned under shade to simulate natural resting sites. Immature *Cx. thriambus* were collected from the primary study site at Highland Creek in September and October. Immature *Cx. stigmatosoma* collected from winery sedimentation ponds in Kelseyville, CA (38.9933 N, −122.8753 W), were used as positive diapause controls (Nelms et al. 2013). *Culex thriambus* and *Cx. stigmatosoma* immatures were reared separately, under natural light and temperature inside the mosquito house in 33 by 23 by 5 cm (L by W by H) white enameled pans containing 1.5 liter of Highland Creek or winery pond water, respectively, until pupation. Emerging adults were partitioned into cohorts based on species and emergence date. A weather logger (UA-002-08 HOBO Pendant, Onset Computer Corp., Bourne, MA) recorded ambient conditions inside the mosquito house. Adult mosquitoes were held in 1-gal (17.0 by 16.8 [Dia. by H] cm) paper cartons covered with window screen and offered a 10% sucrose solution on cotton balls for the duration of the overwintering period. Biweekly, 10–15 females from cohorts with >100 females were dissected to track changes in the morphology of the ovarian follicles. Only 5–10 females were dissected from cohorts with <100 females. Females were sampled on a weekly basis after noticeable changes were observed in the primary follicles following the winter solstice until the deposition of yolk granules (Stage I-II to IIb) in the majority of primary follicles or degeneration of the primary follicles indicated the termination of diapause.

Dissection Protocol

Ovarian dissection procedures were modified from Giglioli (1963) and described previously (Nelms et al. 2013). Briefly, the ovaries were excised with forceps into a drop of distilled water under a dissecting microscope. For field collected females, one ovary was rinsed and dried on a template microscope slide and viewed under a phase contrast compound microscope at 100× to determine parity by examining coiling of the ovarian tracheoles (Detinova 1962). The second ovary was placed in a small drop of 1:1 of Gentian Violet and physiological saline (0.9%) and disrupted using minutin pins for examination of individual ovarioles. For seminatural studies, both ovaries were used for examination of individual ovarioles. Individual follicles were examined at 200× and 400× and the primary follicles were classified morphologically by size and the degree of vitellogenesis in the most mature follicles (Kawai 1969, Clements and Boocock 1984). The lengths of five representative primary and secondary follicles were measured at 200×. The length of the primary follicle was measured from the base of the ovariole to the joining of the secondary follicle. The length of the secondary follicle was then measured to the distal tip of the germarium. The primary follicle length, the ratio of the primary to secondary follicle length, and stages of follicle development were used to determine ovarian status (Spielman and Wong 1973, Reisen et al. 1986). Fully degenerated follicles (>stage II) and the follicles of parous females were not measured.

Follicles in diapausing mosquitoes were at stage I (stages N-Ib of Kawai 1969), whereas females in host seeking arrest were at stage II (stage I-II, IIa,b). To determine if mating had occurred, spermathecae were removed, crushed using forceps in a drop of distilled water and spermatozoa visualized under a compound microscope at 400× to determine insemination.

Bloodmeal Identification

Blood-fed *Cx. thriambus* females were collected opportunistically throughout Lake County by vacuum aspiration from permanent (underground spillways and tunnels) and semipermanent (large resting boxes) resting sites from July 2014 to September 2015. Mosquito abdomens were analyzed to determine the source of the bloodmeal using the methodology of Thiemann et al. (2012). Briefly, DNA was extracted from each abdomen using the DNeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA), and the mitochondrial gene cytochrome *c* oxidase I (*COI*) was amplified from each sample by nested PCR. First, primers flanking *COI* were used to amplify the entire gene, and then the 658-bp "barcoding" region was amplified using vertebrate-specific primers (Ivanova et al. 2006, Cooper et al. 2007). Host DNA was identified by sequencing the *COI* gene and using the "Identify Specimen" feature of the Barcode of Life Data Systems (BOLD; www.boldsystems.org).

Statistical Analysis

The lengths of five primary and secondary follicles were averaged per female and used to calculate the length of the primary follicle and the primary to secondary follicular ratio for each female. Field and seminatural data on follicle stage, primary follicle length, and follicular ratio from dissected *Cx. thriambus* and *Cx. stigmatosoma* were tested for significant effects among species and seasons (prewinter solstice and postwinter solstice) by general linear model-analysis of variance (GLM-ANOVA). Tukey's HSD (honestly significant difference) multiple-comparison tests were used to determine which means were significantly different from one another at the 0.05 significance level. Diapause termination dates were delineated when >50% of the females dissected had terminated diapause. Temperature data gathered from a weather station in Kelseyville (38.9567 N, −122.8917 W), 1.5 km from the primary spillway, and downloaded from Lake County Pear and Grape PestCast Network (http://www.westernwx.com/lakeco/) were used to chart mean daily air temperatures. Temperature data from the weather logger inside the mosquito house were used to calculate cumulative degree-days (°D) in seminatural studies. Degree-day values were calculated using the horizontal cut-off method, single sine model, and the winter solstice as the summation start date using methods available at the University of California Integrated Pest Management Web site (http://www.imp.ucdavis.edu). A lower threshold of 8 °C was chosen as the minimum developmental point estimate based on previous laboratory studies using *Cx. tarsalis* (Reisen et al. 1992).

To test for differences in the proportions of *Cx. tarsalis* and *Cx. thriambus* females captured by vacuum collection compared with proportions captured by CO_2 collection per trap night during the same sampling period, the chi-square test for association was used. All statistical analyses were done using Minitab (2014). Bias-corrected maximum likelihood estimates of virus infection rates (IR) per 1,000 *Cx. thriambus* mosquitoes and 95% confidence intervals (CI) were calculated using PooledInfRate: a Microsoft Excel Add-in (Biggerstaff 2003).

Results

Adult Seasonal Abundance and Dominance

In total, 3,652 females and 884 males representing 12 species were vacuum collected on 70 mornings from January 2014 through December 2015. The same 12 species were collected each sampling year, and there was no significant difference in the mean mosquito abundance for males (paired *t*-test, $t = 1.46$; $P = 0.172$) and females (paired *t*-test, $t = 2.01$; $P = 0.069$) between years, so results from 2014 and 2015 were combined. The seven codominant female species were *Cx. thriambus*, *Culiseta incidens* (Thomson), *Culex apicalis* Adams, *Cx. stigmatosoma*, *Culex boharti* Brookman and Reeves, *Cx. tarsalis*, and *Anopheles punctipennis* (Say) and represented 90.2% of the total collected (Table 1). Female *Cx. thriambus* were the most dominant species and represented 23.1% of the total. There was less diversity among male mosquitoes with five codominant species (*Cs. incidens*, *Cx. thriambus*, *Anopheles franciscanus* McCracken, *Cx. tarsalis*, and *Cx. stigmatosoma*) that represented 90.2% of the total collected (Table 1). *Culiseta incidens* males were the most dominant (38.2%), followed by *Cx. thriambus* (35.1%).

Adult seasonal abundance of *Cx. thriambus* in the vacuum sample is shown for 2014 and 2015 (Fig. 1). Outflows from Highland Springs Reservoir prevented sampling from March through May 2014 and December 2014 through January 2015. During winter 2014, *Cx. thriambus* females were more abundant in January than February. Summer and fall detections of males and females occurred from 6 June through 28 November, with peak abundance on 25 September (46 males and 63 females) and 14 November (61 females). During 2015, *Cx. thriambus* mosquitoes were collected from 11 June through 12 December. The number of adults per trap night peaked on 28 August (22 males and 15 females), then declined steadily until reaching zero on 9 December.

Table 1. Adult female and male mosquitoes collected by vacuum aspiration from Highland Springs Dam during 2014 and 2015

Species	Total	% Total
Females		
Culex thriambus	843	23.1
Culiseta incidens	715	19.6
Culex apicalis	512	14.0
Culex stigmatosoma	357	9.8
Culex boharti	351	9.6
Culex tarsalis	261	7.1
Anopheles punctipennis	254	7.0
Culiseta particeps	178	4.9
Anopheles franciscanus	88	2.4
Anopheles freeborni	77	2.1
Culex territans	14	0.4
Culiseta inornata	2	0.1
Males		
Culiseta incidens	338	38.2
Culex thriambus	310	35.1
Anopheles franciscanus	55	6.2
Culex tarsalis	55	6.2
Culex stigmatosoma	39	4.4
Culiseta particeps	35	4.0
Anopheles punctipennis	23	2.6
Anopheles freeborni	19	2.1
Culex apicalis	8	0.9
Culiseta inornata	2	0.2

Columns show total numbers collected for each species and percentage of total.

For CO_2 trap collections, 279 female mosquitoes representing 13 species were collected over 11 trap nights from 28 August through 6 November 2015 (Table 2). The four codominant species were *Culiseta particeps* (Adams), *Cs. incidens*, *Cx. tarsalis*, and *Cx. thriambus* and represented 88.4% of the total collected. *Culiseta particeps* was the most dominant species and represented 32.1% of the total. A chi-square test showed that there was a significant difference ($\chi^2 = 50.06$, df = 1, $P < 0.001$) in the proportion of *Cx. tarsalis* and *Cx. thriambus* females collected per trap night in CO_2 traps compared to vacuum collections, during the same sampling period. Significantly more *Cx. tarsalis* were collected by CO_2 trap than vacuum collected and the inverse was true for *Cx. thriambus*.

Immature Seasonal Abundance and Dominance

A total of 7,624 mosquito immatures comprising eight species were collected during 2015 (Table 3). *Culex thriambus* was the dominant species, representing 85.1% of the total. Two mosquito species, *Cx. thriambus* and *Cs. incidens*, composed 90.6% of the total immatures collected. Immatures were detected from 4 June through 4 December. The number of immatures per dip peaked on 15 September (287 per dip) and remained relatively abundant until 19 November. No immatures were collected after 4 December.

Diapause Potential and Mating

For each nulliparous, unengorged (stage I-II) *Cx. thriambus* female collected from the primary spillway of Highland Springs Dam during the winters of 2014 and 2015, the primary follicle length, the primary to secondary follicular ratio, and the stages of follicular development are summarized by season in Fig. 2A–C. Winter collections during each sampling year were sporadic due to outflows from the primary spillway, so limited overwintering data for 2014 and 2015 were combined. A total of 591 unfed and 13 gravid females were collected during October through February and of these 169 were dissected to determine diapause status. No blood-fed females were collected during the winter period. Unfed *Cx. thriambus* were grouped by seasons (prewinter solstice, postwinter solstice) and the three ovarian metrics compared (primary follicle length, ratio of primary to secondary follicles, and follicle stage). Means of all three morphometrics were significantly smaller prewinter solstice than postwinter solstice (mean primary follicle length, $F = 85.77$; df = 1; $P < 0.001$; mean ratio, $F = 29.38$; df = 1; $P < 0.001$; mean follicle

Table 2. Adult female mosquitoes collected near Highland Springs Dam by CO_2 trap from 28 August through 6 November 2015

Species	Total no./trap night	% Total
Culiseta particeps	89.5	32.1
Culiseta incidens	65.5	23.5
Culex tarsalis	63.0	22.6
Culex thriambus	28.5	10.2
Culex erythrothorax	16.5	5.9
Anopheles franciscanus	6.0	2.2
Anopheles punctipennis	3.0	1.1
Culex pipiens	2.0	0.7
Culex boharti	1.5	0.5
Culiseta inornata	1.5	0.5
Anopheles freeborni	1.0	0.4
Culex apicalis	0.5	0.2
Culex stigmatosoma	0.5	0.2

Columns show total numbers collected per trap night for each species and percentage of total.

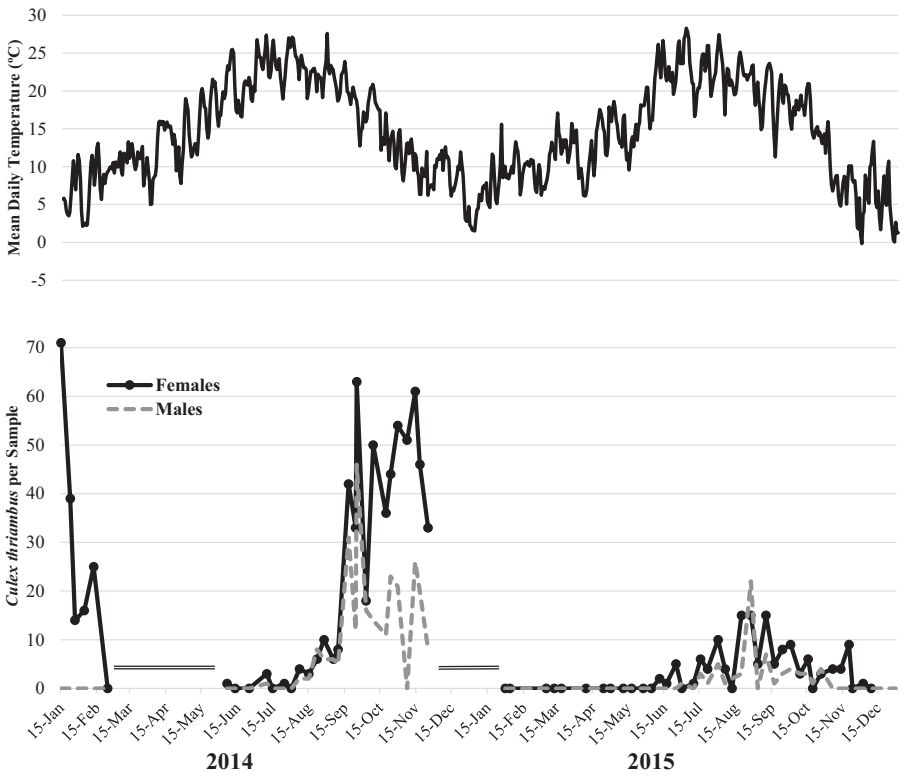

Fig. 1. Abundance and seasonality of male and female *Cx. thriambus* adults collected weekly by vacuum sampling from inside Highland Springs Dam in Lake County, CA, during 2014 and 2015. Double horizontal lines indicate no sampling was conducted due to outflows from Highland Springs Dam. Also shown are corresponding mean daily temperatures.

Table 3. Mosquito immatures collected from Highland Creek in weekly samples from January to December 2015

Species	Total	% Total
Culex thriambus	1,297.0	85.1
Culiseta incidens	85.0	5.6
Culiseta particeps	67.4	4.4
Culex stigmatosoma	36.0	2.4
Culex boharti	22.2	1.5
Anopheles punctipennis	9.0	0.6
Culex apicalis	8.0	0.5
Anopheles freeborni	0.2	<0.1

Columns show mean numbers collected per dip for each species and percentage of total.

stage, $F = 6.79$; df = 1; $P = 0.01$). Overall, 85.8% (145/169) of *Cx. thriambus* females dissected from October through February exhibited nulliparity and were determined to be in a state of ovarian diapause. Of the remaining females, 7.1% (12/169) were in host-seeking arrest (stage II) and 7.1% (12/169) were parous, having uncoiled ovarian tracheoles and follicles with one or more dilatations. Most *Cx. thriambus* females dissected during winter were inseminated, with 90.4% (150/166) containing spermatozoa in their spermathecae.

For seminatural studies, the three ovarian morphometrics were evaluated for *Cx. stigmatosoma* and *Cx. thriambus* (Fig. 2D–F). Overall, 86.2% (50/58) of *Cx. thriambus* and 88.6% (31/35) of *Cx. stigmatosoma* emerging under seminatural conditions in the mosquito house during October entered reproductive diapause, whereas those that emerged in September were a mix of diapausing and nondiapausing forms and were removed from the study. As in field studies, means of all three morphometrics for *Cx. thriambus* (mean primary follicle length, $F = 36.99$; df = 1; $P < 0.001$; mean ratio, $F = 57.23$; df = 1; $P < 0.001$; mean follicle stage, $F = 8.90$; df = 1; $P = 0.003$) and *Cx. stigmatosoma* (mean primary follicle length, $F = 69.98$; df = 1; $P < 0.001$; mean ratio, $F = 69.91$; df = 1; $P < 0.001$; mean follicle stage, $F = 9.40$; df = 1; $P = 0.003$) were significantly smaller prewinter solstice than postwinter solstice. For mean primary follicle length and mean ratio, there was a significant interaction between species and sampling seasons (mean primary follicle length, $F = 9.20$; df = 1; $P = 0.003$; mean ratio, $F = 11.71$; df = 1; $P = 0.001$). For mean follicle stage, there was no significant interaction between species and sampling seasons. Significant comparisons among species and seasons were evaluated using Tukey's HSD and are shown in Fig. 2D–F. More than 50% of *Cx. thriambus* and *Cx. stigmatosoma* females held under seminatural conditions terminated diapause by 30 March (12.9 °C min, 36.7 °C max, 12.6 h day length) and 22 March (8.8 °C min, 28.3 °C max, 12.2 h day length), respectively. The median termination of diapause by *Cx. thriambus* females on 30 March corresponded to an accumulation of 679.51°D, whereas the median termination by *Cx. stigmatosoma* females occurred on 22 March, 1 wk earlier, after 586.03°D (average = 632.77 ± 46.7°D).

Host Selection

A total of 27 blood-fed *Cx. thriambus* females were collected from three underground locations and two large resting boxes in Lake County during 2014 and 2015. Nine were collected from the primary spillway at Highland Springs Dam, 12 from the primary spillway at Adobe Creek Dam (38.9428 N, −122.8919 W), two from Lyons Creek underpass (39.1118 N, −122.9085 W), three from a large resting box in Kelseyville (38.9925 N, −122.8740 W), and one from a large resting box in Lakeport (39.0232 N, −122. 9209 W). All 27 bloodmeal hosts were identified and hosts represented 16 different bird species (Table 4). Of these, 92.6% (25/27) were from the Order Passeriformes. The majority of hosts ($n = 22$, 81.5%) were birds of medium body length (15–20 cm) and year-round residents (Sibley 2000). During June to September ($n = 22$), Western Scrub-Jay (27.3%), Western Bluebird (13.6%), and House Finch (13.6%) composed the majority of *Cx. thriambus* feeds. No blood-fed *Cx. thriambus* females were collected during October through February. A renewal of blood-feeding activity occurred in the spring (March–May) when five blood-fed females were collected that had each fed upon a different passerine bird species. Overall, the most frequently fed-upon host was Western Scrub-Jay, which accounted for 25.9% (7/27) of the total bloodmeals.

WNV Detection

Culex females collected and tested for WN viral RNA from Highland Springs Recreation Area during June through November for 2014 and 2015 are summarized in Table 5. *Culex thriambus* accounted for 56.8% (563/992) and 41.0% (124/306) of the total mosquitoes collected by vacuum aspiration that were tested in 2014 and 2015, respectively. West Nile viral RNA was detected in three pools of *Cx. thriambus* that were vacuum collected on 3 October and 25 November 2014, and 10 September 2015. Infection rates were 3.53 in 2014 and 8.37 in 2015. Only one *Cx. tarsalis* pool was positive on 31 July 2014 (IR = 7.09) and two *Cx. stigmatosoma* pools were positive, one on 15 August 2014 (IR = 3.50) and one on 28 August 2015 (IR = 13.64). A total of 121 *Culex* mosquitoes were tested from two CO_2 traps set out during 28 August through 6 November 2015. Of these, 34 *Cx. thriambus* in six pools were tested with one pool positive for WN viral RNA that was collected on 24 September 2015 (IR = 28.15).

Discussion

Although *Cx. thriambus* mosquitoes are competent vectors for WNV, the principal question is whether they are important in the maintenance, amplification, and overwintering of the virus in nature. To answer this question, important information such as the seasonality, relative abundance, ability to enter a reproductive diapause, host preferences, and potential of *Cx. thriambus* mosquitoes to transmit WNV are needed. To date, little is known about *Cx. thriambus*, so our study evaluated the bionomics and vector potential of this mosquito species within a riparian habitat.

Adult mosquito abundance patterns in California vary among regions, with *Cx. tarsalis* and *Cx. pipiens* complex adults remaining relatively low through May before reaching a peak in July in the Sacramento River Hydrologic Region, which encompasses much of Lake County (Barker et al. 2010). These findings concur with studies of adult female *Cx. stigmatosoma* and *Cx. tarsalis* in Lake County, which found that females of both species were collected from May through October with peak abundance in July and August, respectively (Ryan et al. 2013). In contrast, *Cx. thriambus* adults and immatures were notably more abundant during late summer and fall (September–November) and were rarely collected before June. At northern latitudes, *Cx. tarsalis* pupae can be collected from April through November (Moore 1963, Spadoni et al. 1974), whereas *Cx. thriambus* immatures were collected from June through December. Perhaps the appearance of *Cx. thriambus* immatures at our study site in early June coincides with the delayed emergence of

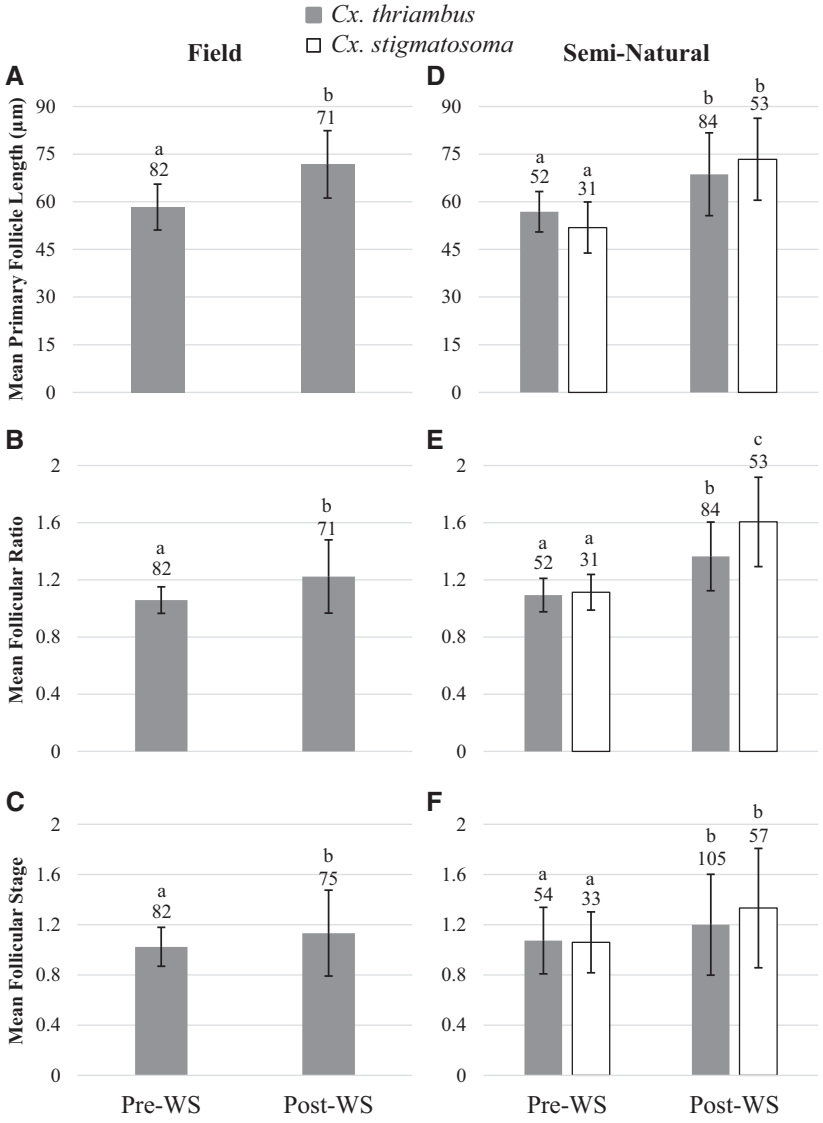

Fig. 2. Seasonal changes (prewinter solstice [Pre-WS] and postwinter solstice [Post-WS]) in the means (± SD) of primary follicle length (**A**, **D**), follicular ratio (**B**, **E**), and follicle stage (**C**, **F**) among field-collected *Cx. thriambus* females and field-collected *Cx. thriambus* and *Cx. stigmatosoma* females reared and held under seminatural conditions in a mosquito house from Highland Springs Dam in Lake County, CA. The number above each column represents the total number of mosquitoes evaluated. Groups of columns under similar letters for the same graph were not significantly different by a Tukey's HSD multiple-comparison test ($P > 0.05$).

females from overwintering hibernacula in late March to early April. Concurrent with these observations, blood-fed and gravid *Cx. thriambus* females first appear in large resting boxes throughout Lake County in March.

The low numbers of adult *Cx. thriambus* mosquitoes collected and tested for arboviruses statewide may be biased by current trapping methodologies that rely on CO_2 or gravid attractants. In our 2-yr study, more *Cx. thriambus* specimens were collected by vacuum than *Cx. tarsalis* while the reverse occurred with CO_2 traps. Carbon dioxide has long been used as an attractant for traps targeting host-seeking mosquitoes for mosquito abundance and arbovirus surveillance programs (Gillies 1980). *Culex thriambus* females were collected in CO_2-baited traps during this study, but in low numbers relative both to their larval population and to other female mosquito species, which supports the hypothesis that this collection method underrepresents their presence. One possible explanation for the dearth of *Cx. thriambus* in CO_2-baited traps may be that they lack CO_2 receptors on their maxillary palps, similar to studies comparing the receptors on the maxillary palps of several *Culex* species have found that *Cx. territans*, a frog-biting species that does not readily enter CO_2 baited traps, had fewer CO_2 receptors than *Cx. tarsalis*, *Culex pipiens* L., and *Culex restuans* Theobald (McIver 1970, McIver and Charlton 1970). Although similar work for *Cx. thriambus* has not been done, CO_2 appears to be more important for host finding and discrimination for mosquitoes with more receptors. Targeted surveillance methods aimed at strictly ornithophagic mosquitoes, such as the use of resting traps or CO_2-baited traps hung in tree canopies (Pfuntner et al. 1988, Anderson et al. 2004), may increase the numbers of females captured and submitted for arbovirus testing.

Diapause was induced in >50% of *Cx. thriambus* and *Cx. stigmatosoma* females that emerged in October and were dissected prewinter solstice, whereas those emerging in mid-late September

Table 4. Bloodmeals identified from *Cx. thriambus* females collected throughout Lake County, CA, from March through May and June through September in 2014 and 2015

			No. of bloodmeals	
			Month	
Order	Species	Common name	Mar.–May	June–Sept.
Accipitriformes	*Buteo lineatus*	Red-shouldered Hawk		1
Passeriformes	*Aphelocoma californica*	Western Scrub-Jay	1	6
	Hirundo rustica	Barn Swallow		2
	Baeolophus inornatus	Oak Titmouse		1
	Psaltriparus minimus	Bushtit	1	
	Polioptila caerulea	Blue-gray Gnatcatcher		1
	Chamaea fasciata	Wrentit		1
	Sialia mexicana	Western Bluebird		3
	Sturnus vulgaris	European Starling	1	
	Pipilo maculatus	Spotted Towhee		1
	Pipilo crissalis	California Towhee		1
	Spizella passerina	Chipping Sparrow		1
	Zonotrichia atricapilla OR leucophrys	Golden OR White-crowned Sparrow	1	
	Agelaius phoeniceus	Red-winged Blackbird	1	
	Haemorhous mexicanus	House Finch		3
Galliformes	*Gallus gallus*	Chicken		1
		Totals	5	22
		%	18.5	81.5

Number and percentage of bloodmeals identified from each bird species by month.

Table 5. Mosquito species collected and tested for West Nile viral RNA from Highland Springs Recreation Area during June through November 2014–2015 (Vacuum) and August through November 2015 (CO_2)

Species	No. confirmed WNV+ pools	No. of pools tested	No. of specimens tested	IR[a] (95% CI)
2014				
Culex stigmatosoma	1	13	282	3.50 (0.21–17.10)
Culex tarsalis	1	7	147	7.09 (0.41–37.16)
Culex thriambus	2	21	563	3.53 (0.65–11.45)
2015				
Vacuum				
Culex pipiens	0	1	1	0 (0.00–0.00)
Culex stigmatosoma	1	7	76	13.64 (0.80–70.71)
Culex tarsalis	0	8	105	0 (0.00–27.82)
Culex thriambus	1	15	124	8.37 (0.48–42.05)
CO_2				
Culex erythrothorax	0	5	18	0 (0.00–130.87)
Culex pipiens	0	1	1	0 (0.00–0.00)
Culex tarsalis	0	5	68	0 (0.00–33.93)
Culex thriambus	1	6	34	28.15 (1.77–131.97)

[a] Bias-corrected maximum likelihood estimates of WNV infection rates.

were a mix of diapausing and nondiapausing forms. Previous studies have also shown that *Culex* females emerging on or shortly after the autumnal equinox exhibit a mixed response compared to those emerging in late winter under short photoperiods and from cool water temperatures (Spielman and Wong 1973, Reisen et al. 1995, Nelms et al. 2013). Follicular observations for *Cx. thriambus* were similar to those described previously for diapausing *Culex* females (Spielman 1974, Eldridge and Bailey 1979, Reisen et al. 1986, Eldridge 1987, Vinogradova 2000, Farajollahi 2005, Nelms et al. 2013). For *Cx. thriambus* held under field and seminatural conditions and dissected prewinter solstice, the mean primary follicle length was ≈57 µm, the mean follicular ratio was 1.1, and no yolk deposition was observed in the majority of primary follicles.

Overall, diapause termination in *Cx. thriambus* and *Cx. stigmatosoma* was concurrent and limited by temperature, with an average of 632.77 ± 6.7°D necessary for termination in at least 50% of populations. This average is similar to results reported for *Cx. pipiens* in New Jersey, where exit from hibernacula was delayed until April–May after ≈700°D (Farajollahi 2005). More °D accumulations may be required for diapause termination in species at northern latitudes where freezing temperatures can occur from late winter into spring. Cessation of diapause is attributed to temperature accrual following the winter solstice, which terminates hormonal constraints on *Culex* ovarian development, and is delayed in more northern compared to southern latitudes (Bellamy and Reeves 1963, Nelson 1971, Reisen 1995, Reisen et al. 1995, Farajollahi 2005, Nelms et al. 2013). Degenerate follicles were observed in the majority of *Cx. thriambus* females immediately following diapause termination, whereas *Cx. stigmatosoma* advanced follicles to host-seeking arrest (stage II). This accounts for the significantly higher mean follicular ratio, postwinter solstice, seen in *Cx. stigmatosoma* held under seminatural conditions. Perhaps if *Cx. thriambus* females do not immediately imbibe a bloodmeal following termination, they proceed with follicular reabsorption of the primary follicles. This phenomenon has been observed previously in *Cx. pipiens* and is attributed to continual secretion of juvenile hormone from the corpora allata (Spielman and Wong 1973, Nelms et al. 2013). Additionally, *Cx. thriambus* females held in small cages under seminatural conditions were exposed to males throughout the entire study, but no females contained sperm in their spermathecae. In contrast, 90.4% of wild, field-collected *Cx. thriambus* females that were dissected during the winter period were inseminated. This suggest that *Cx. thriambus*, like *Cx. tarsalis* and *Cx. stigmatosoma*

(Reisen 2012, Nelms et al. 2013), are eurygamous (mating occurs in swarms in large open spaces).

Host selection results agreed with previous findings that identified bloodmeals using precipitin antisera, documenting that *Cx. thriambus* females primarily feed on competent avian hosts (Tempelis and Reeves 1964). In that study, passerine birds accounted for 90.1% of the bloodmeals identified. The other bloodmeals were from nonpasserine birds (6.6%) and rabbits (3.3%). Mosquito bloodmeal identification methods in the present study found that Western Scrub-Jay, Western Bluebird, House Finch, and Barn Swallow were the most fed upon hosts. Western Scrub-Jays and House Finches have been shown to be especially susceptible to WNV infection and develop extremely high viremias (Komar et al. 2003; Reisen et al. 2005, 2006c). Of the 16 host species, only 7.4% of bloodmeals were from nonpasserine birds (one each from a Red-shouldered Hawk and Chicken) which are considered to be weakly competent or incompetent for WNV (Wheeler et al. 2009). Ornithophagic mosquitoes that feed frequently on competent avian hosts may not serve as bridge vectors of WNV to humans, but play an important role as amplification vectors among passeriform birds (Reisen et al. 2006b). Although *Cx. thriambus* females examined in this study appear to be strictly ornithophagic, limited precipitin data indicates they may also rarely feed on mammals (Tempelis and Reeves 1964). Further bloodmeal data are needed to determine if *Cx. thriambus* females can serve as bridge vectors of WNV to humans.

West Nile virus detections in mosquito pools in September through November coincided with seasonality data. Statewide, *Cx. thriambus* mosquitoes only accounted for 0.3% of the *Culex* mosquito pools tested during 2014 and 2015. The most frequently tested species for WNV from California in 2014 and 2015 were *Cx. tarsalis* (37.3%) and *Culex quinquefasciatus* Say (36.6%), respectively (VBDS Annual Reports; http://www.cdph.ca.gov). Although relatively few *Cx. thriambus* mosquitoes were tested for WNV in California each year, their WNV infection rates were comparable to or higher than the other *Culex* species submitted. Statewide, there were eight WNV confirmed pools of *Cx. thriambus* in 2014 through 2015 and the current study accounted for 62.5% (5/8) of positives (CalSurv Gateway; http://gateway.calsurv.org/). In this study, West Nile viral RNA was detected in a pool containing 11 females collected on 25 November 2014. Notably, 10 of these females were nulliparous and in diapause and one was parous. These results might indicate an instance of horizontal or vertical transmission. Transovarial transmission (the passage of virus from an infected female to progeny) of WNV to nulliparous, overwintering *Culex* has been documented previously (Nasci et al. 2001, Bugbee and Forte 2004, Farajollahi 2005, Andreadis et al. 2010, Unlu et al. 2010). As *Culex* females utilize multiple overwintering strategies, parous females comprise a small portion of the overwintering population regardless of latitude and temperature (Nelson 1964, Kliewer et al. 1969, Mitchell 1979, Andreadis et al. 2010, Nelms et al. 2013) and may serve as horizontal vectors. These data indicate that infected, overwintering *Cx. thriambus* females may be able to initiate WNV transmission in the late winter or spring when temperatures are conducive for host seeking.

In summary, *Cx. thriambus* mosquitoes were effectively sampled by vacuum aspiration of manmade resting sites, although novel trapping methods should be developed and targeted at this and other ornithophagic species. The current study indicated that adults and immatures may be relatively abundant along riparian corridors and that adults are frequently infected with WNV in nature. Members of the *Cx. pipiens* complex and *Cx. tarsalis* are the most important vectors of WNV in California. However, *Cx. pipiens* populations are very low throughout Lake County, with an average of ~70 *Cx. pipiens* complex females collected per season (June–October) in CO_2 and resting traps combined. *Culex tarsalis* are nearly ubiquitous and very abundant in the county; however, they too were relatively scarce at the study site. *Culex thriambus* may be implicated in WNV transmission along riparian systems in the relative absence of other vector-competent *Culex* species. Riparian corridors have been shown to be sites of WNV introduction and subsequent amplification in southern California (Reisen et al. 2004), and *Cx. thriambus* females may be involved as they have been found naturally infected in Los Angeles County (Reisen et al. 2006a). Late-season collections and corresponding positive mosquito pools indicate that *Cx. thriambus* females may also play a role in the overwintering of WNV in California. Overall, this study advocates for continued collection and testing of *Cx. thriambus* mosquitoes to further elucidate their role in the amplification, maintenance, and overwintering of WNV in California.

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