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 (CO₂) 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-km² 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 CO₂ 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 CO₂ 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 2013 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 *Culex 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 *Culex 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 by 8 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 minuten 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 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 (preswimer solstice and postswimer 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 CO2 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, Caliseta 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). Caliseta 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 2015, so results 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    |
| Caliseta 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**                |       |         |
| Caliseta incidens        | 338   | 38.2    |
| Culex thriambus          | 310   | 33.1    |
| 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 CO2 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 CO2 traps compared to vacuum collections, during the same sampling period. Significantly more Cx. tarsalis were collected by CO2 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...
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, at 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

Cx. 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. Cx. thriambus accounted for 36.8% (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 Cx. mosquitoes were tested from two CO2 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
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 CO2 or gravid attractants. In our 2-yr study, more Cx. thriambus specimens were collected by vacuum than Cx. tarsalis while the reverse occurred with CO2 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 CO2-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 CO2-baited traps may be that they lack CO2 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 CO2 baited traps, had fewer CO2 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, CO2 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 CO2-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

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).
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

| Order         | Species                  | Common name                  | Month          | No. of bloodmeals |
|---------------|--------------------------|------------------------------|----------------|-------------------|
| Passeriformes | Aphelocoma californica   | Western Scrub-Jay            | Mar.–May       | 1                 |
|               | Hirundo rustica          | Barn Swallow                 | June–Sept      | 6                 |
|               | Baeolophus inornatus     | Oak Titmouse                 |                |                   |
|               | Psaltriparus minimus      | Bushtit                      |                |                   |
|               | Polioptila caerulea      | Blue-gray Gnatacatcher       |                |                   |
|               | Chimarrae fasciata       | Wrentit                      |                |                   |
|               | Stilts mexicana          | Western Bluebird             |                |                   |
|               | Sturnus vulgaris         | European Starling            |                |                   |
|               | Pipilo maculatus         | Spotted Towhee               |                |                   |
|               | Pipilo crissalis         | California Towhee            |                |                   |
|               | Spizella passerina       | Chipping Sparrow             |                |                   |
|               | Zonotrichia atricapilla OR leucophrys | Golden OR White-crowned Sparrow |              | 1                 |
|               | Agelaius phoenicuens     | Red-winged Blackbird         |                | 1                 |
| Galliformes   | Gallus gallus            | House Finch                  |                | 3                 |
|               |                          | Chicken                      |                | 1                 |
|               |                          | Totals                       |                | 5                 |
|               |                          | %                            |                | 18.5              |
|               |                          | No. of bloodmeals            |                | 22                |
|               |                          | No. of specimens tested      |                |                   |
|               |                          | IR* (95% CI)                 |                |                   |
| 2014          | Cx. stigmatosoma         | 1                            | 13             | 282               | 3.50 (0.21–17.10) |
|               | Cx. tarsalis             | 1                            | 7              | 147               | 7.09 (0.41–37.16) |
|               | Cx. thriambus            | 2                            | 21             | 563               | 3.53 (0.65–11.45) |
| 2015          | Cx. pipiens              | 0                            | 1              | 1                 | 0 (0.00–0.00)     |
|               | Cx. stigmatosoma         | 1                            | 7              | 76                | 13.64 (0.80–70.71) |
|               | Cx. tarsalis             | 0                            | 8              | 105               | 0 (0.00–27.82)    |
|               | Cx. thriambus            | 1                            | 15             | 124               | 8.37 (0.48–42.05) |
|               | CO2                      | 0                            | 5              | 18                | 0 (0.00–130.87)   |
|               | Cx. erythrothorax        | 0                            | 1              | 1                 | 0 (0.00–0.00)     |
|               | Cx. pipiens              | 0                            | 5              | 68                | 0 (0.00–33.93)    |
|               | Cx. tarsalis             | 1                            | 6              | 28.15 (1.77–131.97) |

* 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). 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°C 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°C (Farajollahi 2005). More °C 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 suggests that Cx. thriambus, like Cx. tarsalis and Cx. stigmatosoma...
Host selection results agreed with previous findings that identified bloodmeal sources 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 ornithophagous, 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 coincide 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 in 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, Kliever 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 ornithophagous 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 CO2 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. *Cx. 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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