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SEASONALITY AND TIME OF HOST-SEEKING ACTIVITY OF *CULEX TARSALIS* AND FLOODWATER *AEDES* IN NORTHERN COLORADO, 2006–2007[1]

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ABSTRACT. Effective and economical control of adult vector and pest mosquitoes requires knowledge of their seasonal abundance and host-seeking activity patterns. We conducted research in 2006–2007 to study these variables for *Culex tarsalis*, *Aedes vexans*, *Ae. melanimon*, and *Ae. dorsalis* in Larimer County, CO. Mosquitoes were collected with traps that segregated catches in 7 consecutive 2-h intervals initiating at 1730 h at 4 sites. Seasonal abundance varied for all species by site and year. Time of host-seeking activity was consistent for all species by site and year. *Culex tarsalis* counts were significantly higher 1.2–4.5 h after sunset than during the preceding time intervals. Maximum host-seeking activity of the 3 *Aedes* species occurred from 0.8 h before sunset to 6.5 h after. Host seeking by all species continued throughout the night. For optimal control of *Cx. tarsalis* adulticide application should start approximately 1 h after sunset, and control of *Aedes* species should begin soon after sunset, and for all species applications can continue throughout most of the night.

KEY WORDS *Culex tarsalis*, floodwater *Aedes*, seasonality, host seeking, control

INTRODUCTION

The 1st line of defense against vector and pest mosquitoes is the elimination or treatment of larval habitats of the target species. When this technique is insufficient to reduce pest densities or arbovirus transmission, it may become necessary to reduce the adult female mosquito population, generally by ground or aerial application of adulticides. For adulticiding to have an optimal effect it is necessary to have detailed information concerning the biology of the species to be controlled. Especially important in this regard are knowledge of seasonal abundance and diel activity patterns. The time of host-seeking activity is particularly important, as adulticides must directly impact mosquitoes, generally while in flight, to be effective. Although the highest infection rates in vector species, and highest levels of virus transmission to humans, may occur later in the summer when female vector abundance is declining and the population consists primarily of older individuals, amplification of virus in the mosquito population begins earlier in the season as vector abundance is increasing. Monitoring vector populations and infection rates during this time may allow for the detection of increasing levels of virus amplification that would trigger the use of adulticides before human infections are detected (Lothrop et al. 2008).

In northern Colorado, and the western United States in general, *Culex tarsalis* Coq. and *Cx. pipiens* L. are the primary vectors of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) (Goddard et al. 2002, Hayes et al. 2005, Turell et al. 2005, Bolling et al. 2007, Gujral et al. 2007), and of St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*, SLEV) (Cockburn et al. 1957, Reeves 1990, Smith et al. 1993). During 2003, an epidemic year for WNV in Colorado, 2,947 human cases were recorded statewide, with 546 cases occurring in Larimer County in north-central Colorado (http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount03_detailed.htm). *Culex tarsalis* is also the primary vector of western equine encephalitis virus (family *Togaviridae*, genus *Alphavirus*, WEEV) (Reeves 1990), although a secondary transmission cycle of this virus involving *Aedes melanimon* Dyar and black-tail jackrabbits has also been documented (Reisen 1984).

Additionally, a number of floodwater *Aedes* species, such as *Ae. vexans* (Meigen), *Ae. dorsalis* (Meigen), and *Ae. melanimon*, can be significant pests of both humans and livestock (Harmston and Lawson 1967, Pennington and Lloyd 1975), thus generating demands for control efforts to reduce biting intensity. These species also have been found to be naturally infected with WNV (Anderson et al. 2007, Bolling et al. 2007, Cupp et al. 2007), and are competent vectors in laboratory studies (Goddard et al. 2002, Tiawsirisup et al. 2008, Turell et al. 2005). Thus, they should be considered as potential secondary and possible bridge vectors of WNV, although their preference for feeding primarily on mammalian hosts (Tempelis and Washino 1967, Gunstream et al. 1971, Apperson et al. 2004) may limit their effectiveness in those roles.

[1] Opinions expressed are those of the authors and do not necessarily represent the opinions of the Centers for Disease Control and Prevention.

Studies conducted in California utilizing CO_2-baited traps (Berlin et al. 1976, Walters et al. 1979, Meyer et al. 1984, Reisen et al. 1997), or human landing collections (Nelson and Spadoni 1972, Berlin et al. 1976, Walters et al. 1979, Cope et al. 1986), demonstrated that *Cx. tarsalis* begins host-seeking soon after sunset, and remains active for several hours before activity declines or ceases. These observations are supported by laboratory experiments showing that both WEEV-infected and uninfected *Cx. tarsalis* host seek throughout the night, with a peak of activity immediately after complete darkness begins (Lee et al. 2000).

Host-seeking by *Ae. vexans* and *Ae. melanimon* attracted to CO_2-baited traps or human bait in California peaked 1–2 h after sunset (Nelson and Spadoni 1972, Meyer et al. 1986). However, with the use of New Jersey light traps and unbaited Malaise traps, Miura and Reed (1970) found a pronounced crepuscular pattern of *Ae. melanimon* flight and biting activity, although the spike in activity near sunrise may have been due to females searching for diurnal resting sites (Reisen and Reeves 1990). In the midwestern USA, *Ae. vexans* counts in CO_2-baited time-segregated traps peaked between 2100 and 2300 h from May through September, or 1–4 h postsunset, depending upon the time of sunset on a particular trapping date (Mitchell 1982).

Published data on the relevant biology of the species discussed above are lacking for northern Colorado. Thus, we undertook studies during 2006 and 2007 to derive detailed information on the seasonal abundance and host-seeking activity of these species. Our goal was to provide mosquito control decision makers in Colorado and the Great Plains region with information useful for optimizing control measures that will reduce the burden of disease and annoyance caused by mosquitoes.

MATERIALS AND METHODS

Study sites: The study was conducted in Larimer County, CO, one of the most populous counties in northern Colorado. The county comprises approximately 6,838 square kilometers, with an estimated population in 2005 of ~283,000 residents (http://www.co.larimer.co.us/about/about.htm). The county is geographically diverse, varying from semiarid high plains in the east (where most arbovirus transmission and pest mosquito annoyance occurs), to alpine habitat in the western part of the county. The 4 mosquito species under consideration are limited primarily to plains and foothills–low montane areas of the county (Eisen et al. 2008).

Four collection sites were selected to represent different habitat types within Larimer County. An "Urban" site in eastern Fort Collins was a residential neighborhood of single-family homes with mature trees and shrubbery. A "Natural" site was located at the Colorado State University Environmental Learning Center along the Poudre River on the eastern edge of Fort Collins. This site is an ~86-ha research and environmental education area containing 4 ecologic zones: cottonwood forest, riparian, wetland, and prairie. A "Suburban/Rural (Sub/rural)" site located on the northern edge of Fort Collins is a 4.5-ha farm consisting of nonirrigated pasture, with new suburban residential development approximately 0.5 km to the east. A "Rural" site located approximately 25 km northeast of Fort Collins is a 20.6-ha farm, where sheep were raised, with ~8.5 ha under irrigation.

Temperature and rainfall data were obtained from a National Weather Service station in Fort Collins (40°37′N, 105°8′W) (http://www7.ncdc.noaa.gov/IPS/cd/cd.html).

Mosquito collection: Mosquitoes were trapped for 1 night per week from 15 May to 28 September in 2006, and for 2 nights per month (biweekly) from 24 May to 20 September in 2007. At each collection site, 2 Centers for Disease Control (CDC) miniature light traps (Hausherr's Machine Works, Toms River, NJ), baited with 1.1 kg of dry ice, were attached to Collection Bottle Rotators (CBRs) (John Hock Model 1512, Gainesville, FL). Each CBR had seven 0.5-liter plastic collection bottles, with each bottle containing 1/3 of a Vaportape II pesticide strip (Bioquip Products Inc., Rancho Dominguez, CA) to immobilize the mosquitoes. Pesticide strips were replaced at approximately monthly intervals. Collection Bottle Rotators were programmed such that there were seven 2-h collection intervals beginning at 1730 h and ending at 0730 h the next morning. Due to the variation in time of sunset as the season progressed, the relationship of the times of the collection intervals to sunset was different for each collection date. The time of sunset ranged from 2013 h on May 18, 2006, the 1st collection date, to 1848 h on September 28, the last collection date that year, and from 2018 h on May 24, 2007, to 1901 h on September 20, 2007. Thus, the start of the 1730–1930-h collection interval ranged from 2.7 h before sunset at the start of the 2006 season to 1.3 h before sunset at the end of the season. Likewise, for 2007 the corresponding intervals ranged from 2.8 h before sunset at the start of the season on May 24 to 1.5 h before sunset at the end of the season on September 20. During both years, the 1st collection interval started before sunset throughout the trapping season, and the 2nd interval began before sunset early in the season but after sunset by the end of the season. The 2130–2330-h and later intervals always began after sunset. The beginning of the 2130–2330-h interval ranged from 1.2 to 2.7 h postsunset. The

Table 1. Number (%) of specimens collected by collection bottle rotator traps at 4 sites in Larimer County, CO, during 2006 and 2007.

2006		2007	
Species	No. collected (%)	Species	No. collected (%)
Aedes vexans	37,918 (78)	*Ae. vexans*	16,937 (75)
Ae. melanimon	6,348 (13)	*Ae. melanimon*	3,144 (14)
Culex tarsalis	2,149 (4)	*Cx. tarsalis*	1,508 (7)
Ae. dorsalis	712 (2)	*Ae. dorsalis*	417 (2)
Ae. trivittatus	703 (1)	*Ae. increpitus*	340 (1)
Ae. increpitus	615 (1)	*Ae. trivittatus*	117 (<1)
Coquillettidia perturbans	137 (<1)	*Culiseta inornata*	104 (<1)
Cx. pipiens	97 (<1)	*Cq. perturbans*	32 (<1)
Culiseta inornata	71 (<1)	*Cx. pipiens*	32 (<1)
Other species collected[1]	21 (<1)	Other species collected[2]	44 (<1)
Total	48,771 (100)	Total	22,675 (100)

[1] *Aedes hendersoni* (7), *Aedes* species (8), *Anopheles earlei* (2), *An. punctipennis* (1), *Psorophora signipennis* (1), *Culex* species (2).
[2] *Aedes* species (4), *An. earlei* (2), *Culex* species (38).

beginning of the last (0530–0730-h) collection interval ranged from 0.2 to 1.4 h before sunrise.

Collections were returned to the laboratory and stored at −80°C. Mosquitoes were identified and pooled on a refrigerated chill table with the use of the key of Darsie and Ward (2005). Pools were triturated with the use of a Mixer Mill 300 (Qiagen, Valencia, CA) (Nasci et al. 2002), and tested for the presence of WNV RNA by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) (Lanciotti et al. 2000, Nasci et al. 2002).

Statistical analysis: Generalized linear models were fit with mosquito count as the response and number of trap-nights as an offset. A negative binomial model (due to overdispersion in the Poisson fit) was assumed and used with a log link. Standard diagnostics were run to ensure a good fit of the final model. Multiple comparisons were computed with the use of Sidak's method for interaction terms that were statistically significant. In order to identify the peak host-seeking times of each species at each site, counts were regressed on site, species, and time interval, including 2- and 3-way interactions. Differences were considered significant when $P < 0.05$. Multiple comparisons were done with an overall probability for a Type I error of 0.05. Mosquito infection rates (IRs) were calculated as the maximum-likelihood estimate with 95% confidence intervals (CIs) (Biggerstaff 2006).

RESULTS

General results: During the 2-year study, a total of 71,446 mosquitoes were captured in CBR traps; 48,771 during the weekly collections in 2006 and 22,675 during the twice-monthly collections in 2007 (Table 1). In both years the Natural site had the greatest abundance of mosquitoes, with 44,668 (91.6% of the total) collected in 2006, and 17,596 (77.6% of the total) in 2007. The Rural and Sub/Rural sites were the next most abundant, respectively. In 2006 3,114 (6.4% of the total) mosquitoes were collected at the Rural site, and 3,803 (16.3%) were collected in 2007. At the Sub/Rural site 848 (1.7%) mosquitoes were collected in 2006, and 1,249 (5.5%) in 2007. The Urban site had the lowest abundance of mosquitoes in both years, yielding 135 (0.3%) in 2006 and 160 (0.7%) in 2007. In both years, *Ae. vexans* was the most abundant species collected, comprising 78% of the total in 2006 and 75% in 2007. Other commonly collected species included *Ae. melanimon* (13% and 14%, respectively, in 2006 and 2007), *Cx. tarsalis* (4% and 7%), and *Ae. dorsalis* (2% each year). *Culex pipiens* constituted less than 1% of the total mosquitoes collected in both years, with 97 collected in 2006, and 32 in 2007.

Weather patterns during the 2 years were similar (Fig. 1A). Mean monthly precipitation was below the 30-year norm from May through September in both years, except in August 2007 (Fig. 1B). Total precipitation during 2006 was 286.5 mm, and 347 mm during 2007. May–September precipitation accounted for only 29% of the annual total in 2006, but was 51% of the 2007 total. A single large precipitation event (73.7 mm) that occurred on August 2–3, 2007, accounted for most of this difference between years. Mean monthly temperature was at or above the 30-year norm during the May–August period in 2006, but decreased below the norm in September 2006 (Fig. 1B). Temperatures were at or above the norm during May–September 2007. Accumulated cooling degree-days (CDD) using an 18.3°C (65°F) base were similar for the 2 years, with 795 degree-days through September 2006, compared to 819 degree-days through the same period in 2007.

Relative abundance by site: When the relative abundance of a species among sites was examined, *Cx. tarsalis* was most abundant in 2006 at

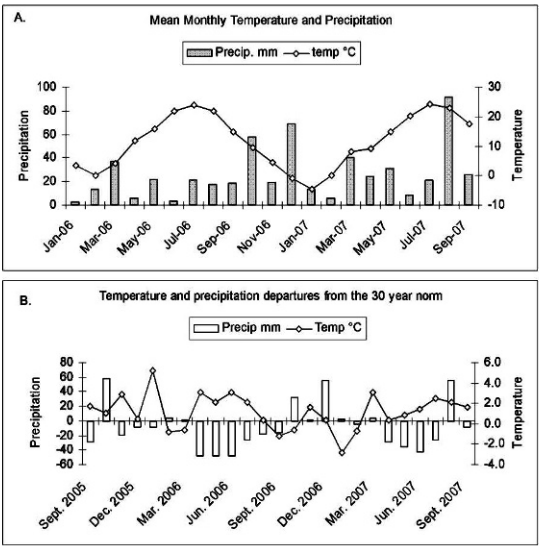

Fig. 1. (A) Mean monthly temperature and precipitation, (B) temperature and precipitation deviations from the 30-year norm. Data obtained from the National Weather Service.

the Natural site (80% of the total collected), followed by the Sub/Rural, Rural, and Urban sites (11%, 6%, and 3%, respectively) (Table 2). In contrast, during 2007 *Cx. tarsalis* was most abundant at the Rural site (42%). Abundances at the Natural and Sub/Rural sites were similar (26% and 27%, respectively), and abundance at the Urban site in 2007 was again lowest at 6%.

Patterns observed for *Ae. vexans* and *Ae. melanimon* were somewhat similar to each other. In 2006, both species were most abundant at the Natural site (94% and 85%, respectively, of the totals collected of those species), followed by the Rural site (5% and 14%, respectively). Relative abundances at the Sub/Rural and Urban sites in 2006 were ≤1% of the totals collected. During 2007, the relative abundance of *Ae. vexans* decreased slightly to 89% at the Natural site and increased to 11% at the Rural site. However, the abundance of *Ae. melanimon* was lower at the Natural site in 2007 (57%) compared to 2006 (85%), and increased at the Rural site to 40% of the total collected. Unlike the other 3 species, *Ae. dorsalis* was most abundant in both years at the Rural site (76% of the total collected in 2006, 55% in 2007) and the Sub/Rural site (16%, 38%), but abundance was low or undetectable at the Natural and Urban sites.

The relative abundance among the 4 species at each site was also compared. *Aedes vexans* was the most abundant species collected at the Natural, Rural, and Sub/Rural sites during 2006 (83%, 52%, and 54%, respectively), and at the Natural and Rural sites in 2007 (87% and 45%, respectively) (Table 2). *Culex tarsalis* was the most abundant of the 4 species collected at the Urban site during both years (52% and 56%, respectively), and at the Sub/Rural site (56%) in 2007, but was the least abundant of the 4 species at the Rural site in 2006 (4% of the total).

Seasonal abundance: The trends in seasonal abundance for all 4 species varied by year and by site. During 2006, *Cx. tarsalis* abundance peaked at 251 females per trap-night (TN) on June 22 at the Natural site, then declined rapidly (Fig. 2). Smaller peaks were observed at this site during July and early August. At the other 3 sites, abundance was generally low, with maximum trap counts for *Cx. tarsalis* at the Rural and Sub/Rural sites of 12.5/TN and 29.5/TN, respectively.

Table 2. Relative abundance of *Culex tarsalis*, *Aedes vexans*, *Ae. melanimon*, and *Ae. dorsalis* at 4 sites in Larimer County, CO, during 2006 and 2007.

	Site				
Species	Natural	Rural	Sub/Rural	Urban	Total
2006					
Cx. tarsalis	43.4[1] (80)[2] *(4)*[3]	3.5 (6) *(4)*	5.9 (11) *(26)*	1.7 (3) *(52)*	54.5 (100)
Ae. vexans	895.2 (94) *(83)*	43.6 (5) *(52)*	12.1 (1) *(54)*	1.3 (<1) *(39)*	952.2 (100)
Ae. melanimon	137.0 (85) *(13)*	21.8 (14) *(26)*	1.5 (1) *(7)*	0.2 (<1) *(6)*	160.5 (100)
Ae. dorsalis	1.5 (8) *(<1)*	14.5 (76) *(17)*	3.1 (16) *(14)*	0.1 (<1) *(3)*	19.1 (100)
Total	1,077.1 *(100)*	83.4 *(100)*	22.6 *(100)*	3.3 *(100)*	
2007					
Cx. tarsalis	10.3 (26) *(2)*	16.7 (42) *(17)*	10.6 (27) *(56)*	2.2 (6) *(56)*	39.8 (100)
Ae. vexans	378.5 (89) *(87)*	44.9 (11) *(45)*	2.0 (<1) *(11)*	1.6 (<1) *(41)*	427.0 (100)
Ae. melanimon	46.6 (57) *(11)*	32.4 (40) *(32)*	2.1 (3) *(11)*	0.1 (<1) *(3)*	81.2 (100)
Ae. dorsalis	0.8 (7) *(<1)*	6.1 (55) *(6)*	4.3 (38) *(23)*	0	11.2 (100)
Total	436.2 *(100)*	100.1 *(100)*	19.0 *(100)*	3.9 *(100)*	

[1] Abundance expressed as the mean number of mosquitoes per trap night.
[2] The distribution of a species across collection sites is calculated as the percentage of that species at all 4 sites (across rows).
[3] The relative proportions of the 4 species at each site are shown in italics, as the percentage of the total by site and year (down columns).

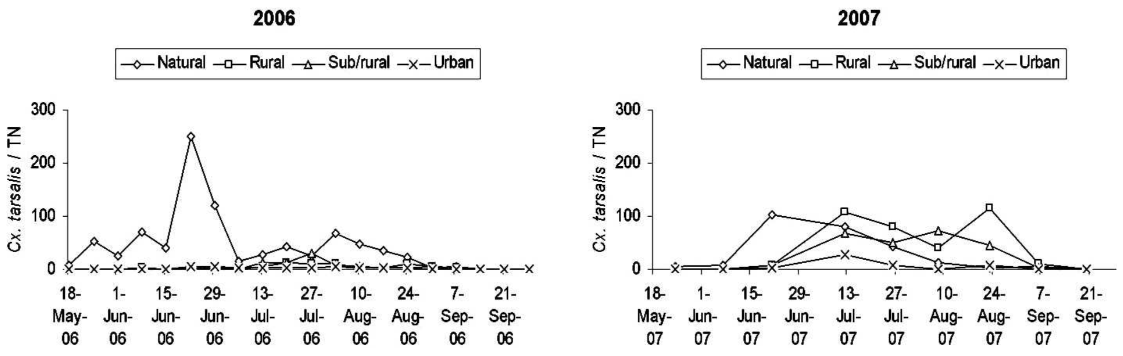

Fig. 2. Seasonal abundance patterns of *Culex tarsalis* at 4 study sites in Larimer County, CO, during 2006 and 2007.

Abundance at the Urban site in 2006 never exceeded 4.5/TN. In 2007, *Cx. tarsalis* abundance at the Natural site peaked at 103/TN on June 21, then declined steadily throughout the summer. However, abundance at the other sites was higher in 2007 than in 2006, and population peaks were observed later in the season than at the Natural site. At the Rural, Sub/Rural, and Urban sites a spike in abundance was seen on July 12, with a 2nd peak observed at the Rural and Sub/Rural sites on August 23 and August 8, respectively.

In 2006, both *Ae. vexans* and *Ae. melanimon* exhibited a sharp spike in abundance on June 8 at the Natural site to 6,205/TN and 1,204/TN, respectively, followed by a rapid decline in numbers collected (Fig. 3). Two smaller secondary peaks were observed in late June and mid-July, then numbers declined to low levels for the remainder of the summer. In 2007, populations of both species increased at the Natural site on June 21, 2 wk later than in 2006, with *Ae. melanimon* abundance continuing to increase until July 12. A smaller increase in abundance was seen on August 23 for *Ae. vexans*, but not for *Ae. melanimon*. These species also showed similar seasonal abundance patterns at the Rural, Sub/Rural, and Urban sites within a year, although the patterns were very different between the 2 yr. During 2006 each species had several peaks in abundance from mid-July to late August. In contrast, during 2007 only a single August peak in abundance was observed at the Rural and Sub/Rural sites. At the Urban site abundance never exceeded 16.5/TN for *Ae. vexans*, and 2.5/TN for *Ae. melanimon*.

Aedes dorsalis abundance at the Natural site was low during both years, never exceeding 5/TN in 2006 and 21/TN in 2007 (Fig. 4). At the Rural site 4 distinct peaks were observed in 2006 between May 25 and August 24, with abundance greatest on the latter collection date (64.5/TN). During 2007, only 2 peaks in abundance were seen, the 1st on June 21 (18.5/TN), and the 2nd from August 8 to August 23 (28/TN and 25/TN, respectively). At the Sub/Rural site abundance was low in both years, with maximum trap counts of 12/TN on August 17, 2006, and 47/TN on August 8, 2007. Counts at the Urban site never exceeded 1/TN in either year.

Host-seeking activity: Mosquitoes were collected during seven 2-h intervals throughout the night beginning at 1730 h. As the patterns of host-seeking activity for all species and sites did not vary significantly between years, data from 2006 and 2007 were combined for analysis. Generally, activity increased for all species at all sites until the 2130–2330-h time interval (1.2–4.7 h postsunset), then declined more or less slowly throughout the night. All species were active throughout the night. A secondary peak in host-seeking activity near sunrise was not observed in either year for any species.

Host-seeking activity by *Cx. tarsalis* increased significantly from the 1st (1730–1930 h) and 2nd (1930–2130 h) collection intervals to the 3rd (2130–2330 h) interval at all sites (Fig. 5). At the Natural and Urban sites activity during the 4th (2330–0130-h) interval was also significantly greater than during the earliest 2 time intervals. *Aedes vexans* activity increased significantly at all sites from the 1st collection interval to the 2nd interval, with a nonsignificant increase during the 3rd interval. By contrast, the number of *Ae. melanimon* collected during the 1st time interval was significantly lower than those in the 2nd interval only at the Rural site. Numbers of *Ae. melanimon* collected during the 3rd interval were not significantly different from those collected during the 2nd at all sites, and did not differ from those collected during the 4th interval at the Natural and Urban sites. Significantly fewer *Ae. dorsalis* were captured during the 1st collection interval than during the 2nd and 3rd time intervals at the Natural and Rural sites, but not at the Sub/Rural site. This species was collected in small numbers at the Urban site. Generally,

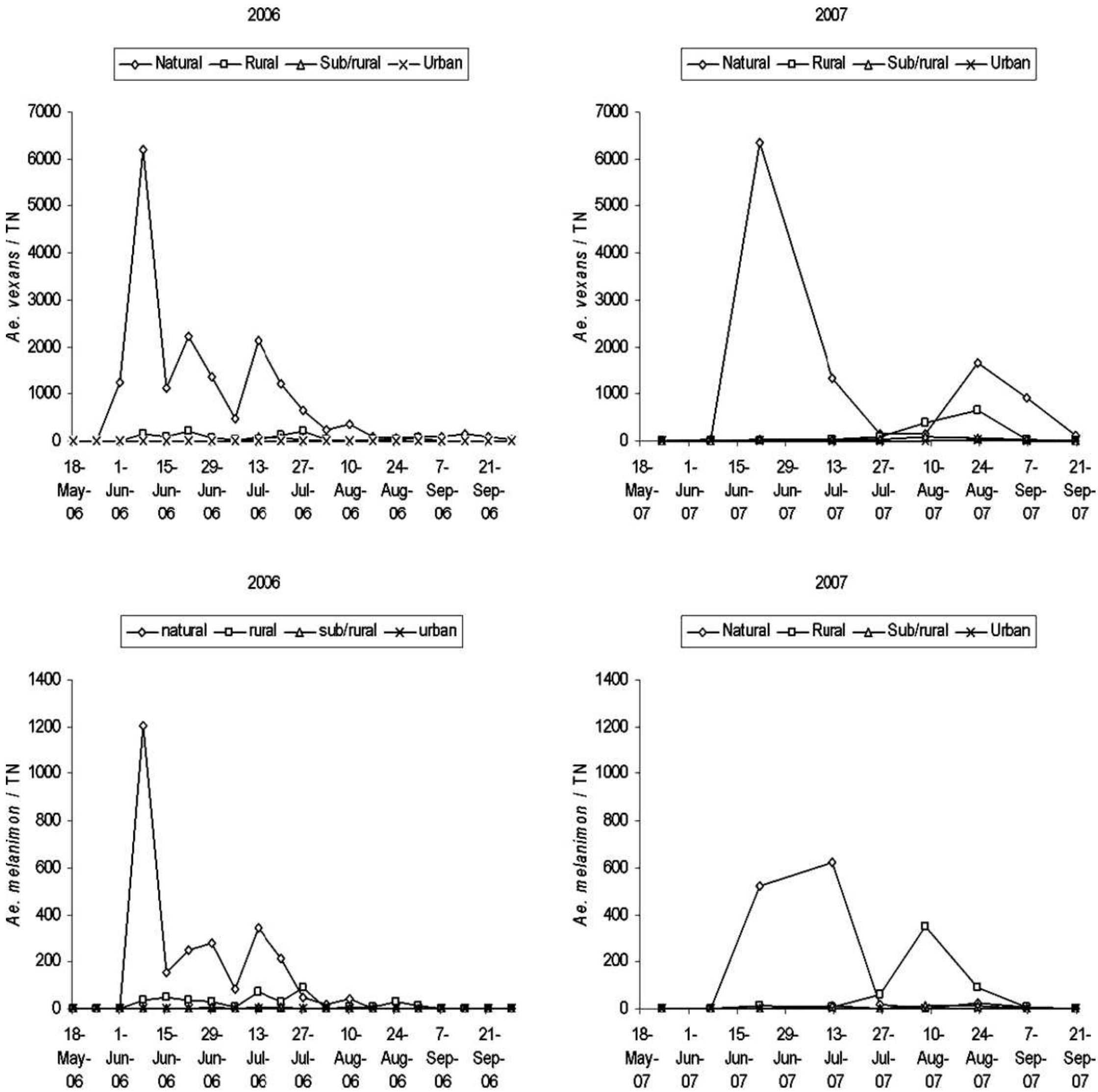

Fig. 3. Seasonal abundance patterns of *Aedes vexans* and *Ae. melanimon* at 4 study sites in Larimer County, CO, during 2006 and 2007.

numbers of *Ae. dorsalis* collected during the 1st interval were not significantly different than those collected during the 4th and later intervals.

It would be of interest to know the host-seeking activity patterns of *Cx. pipiens*; however, only 129 individuals were collected during the study, and this species was not included in Fig. 5. When data were combined for all 4 sites in both years, approximately equal numbers of *Cx. pipiens* females were collected in the 1930–2130-h through 0130–0330-h collection intervals (27–33 females per interval). Only 7 and 4 females were collected in the 0330–0530 h and 0530–0730-h collection intervals, respectively, and none in the 1730–1930-h interval.

West Nile virus activity: During the study a total of 14 WNV-positive mosquito pools were detected, 3 in 2006 and 11 in 2007, including 10 pools of *Cx. tarsalis*, 2 pools of *Ae. melanimon*, and 1 pool each of *Ae. vexans* and Cx. *pipiens*. Five positive pools were from the Natural site (2 from *Cx. tarsalis*, 2 from *Ae. vexans*, and 1 from *Cx. pipiens*), 5 pools from the Rural site (3 *Cx. tarsalis*, 2 *Ae. melanimon*), and 4 pools from the Sub/Rural site (all *Cx. tarsalis*). Positive pools were distributed among collection time intervals as follows: 3 pools from 1930 to 2130 h (1 each from *Cx. tarsalis*, *Cx. pipiens*, and *Ae. vexans*), 5 pools from 2130 to 2330 h (3 *Cx. tarsalis*, 2 *Ae. melanimon*), 2 pools from 2330 to 0130 h (both

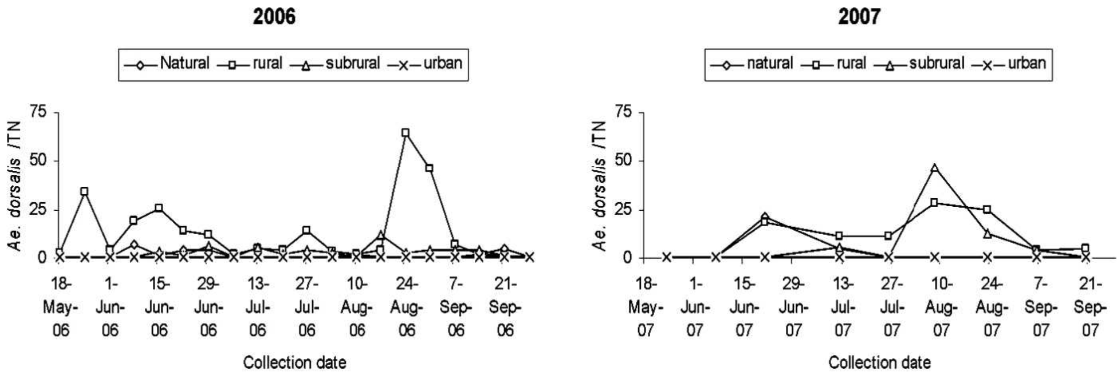

Fig. 4. Seasonal abundance patterns of *Aedes dorsalis* at 4 study sites in Larimer County, CO, during 2006 and 2007.

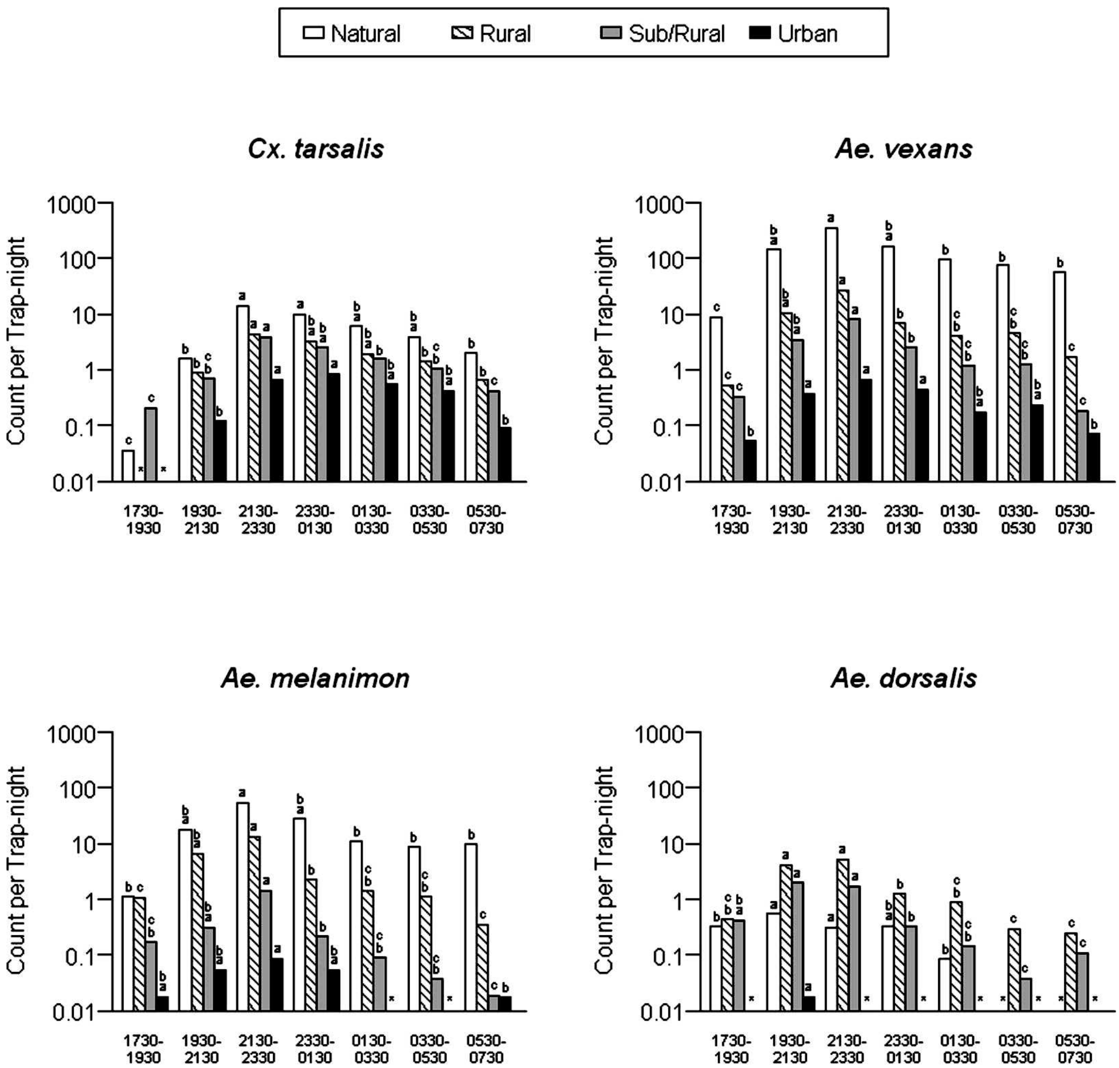

Fig. 5. Host-seeking activity patterns of *Culex tarsalis*, *Aedes vexans*, *Ae. melanimon*, and *Ae. dorsalis* at 4 study sites in Larimer County, CO, during 2006 and 2007. Time of host seeking is presented as the absolute time of collection intervals (e.g., 1730–1930 h). For each species within a study site bars with the same letter are not statistically different ($P > 0.05$).

Cx. tarsalis), 1 pool from 0130 to 0330 h (*Cx. tarsalis*), and 3 pools from 0330 to 0530 h (2 *Cx. tarsalis*, 1 *Ae. vexans*). During 2006, the earliest positive pool was collected on June 6, another on August 24, and the last on September 7. In 2007, the 1st 2 positive pools were from collections made on July 12, and the last 2 from collections on September 6. Two additional pools were collected in July, and 5 pools in August. Combining counts for all sites and both years, overall IRs were 2.79 per thousand (95% CIs = 1.43–4.95) for *Cx. tarsalis*, 0.21 per thousand (0.04–0.70) for *Ae. melanimon*, 0.02 per thousand (0.00–0.09) for *Ae. vexans*, and 7.71 per thousand (0.45–36.57) for *Cx. pipiens*.

DISCUSSION

Seasonal abundance of the 4 species studied varied dramatically between years and study sites. *Culex tarsalis* populations peaked earliest at the Natural site in both years, followed by a decline through the rest of the summer. At the other sites *Cx. tarsalis* abundance peaked later, and at the Rural site a 2nd substantial peak was observed during August 2007. In 2006, the *Cx. tarsalis* abundance peak occurred 2 wk later than the *Ae. vexans* or *Ae. melanimon* peaks at the Natural site, but this difference was not observed in 2007. The Natural site is in the floodplain of the Poudre River on the east side of Fort Collins, and it is likely that the June spikes in *Cx. tarsalis* and floodwater *Aedes* abundance at this site were associated with the creation of freshwater pools resulting from spring snowmelt that hatched overwintering *Aedes* eggs, and attracted gravid *Cx. tarsalis* to oviposit. Supporting this, Reisen and Reeves (1990), summarizing results of earlier studies, showed that *Cx. tarsalis* populations increased rapidly in Kern County, CA, following wet winters and high river flow rates during the spring and early summer. The earliest seasonal peaks were seen at riparian sites, whereas in rural locations seasonal abundance peaks were influenced by irrigation patterns. *Culex tarsalis* overwinter as inseminated females that emerge in spring, bloodfeed, and then search for a suitable water source for oviposition. Thus, *Cx. tarsalis* abundance peaks in riparian areas may follow those of floodwater *Aedes* that overwinter as eggs and hatch when inundated in spring. *Aedes vexans* abundance peaked during early to mid-June at the Natural site in both years, and *Ae. melanimon* numbers peaked in early June during 2006. In 2007, *Ae. melanimon* abundance was lower at the Natural site than in 2006, and the abundance peak extended from mid-June to mid-July. These species had multiple peaks of abundance at the Rural site, but only a single peak at the Sub/Rural site in 2006. In 2007, both species exhibited only a single large spike in abundance during August. *Aedes dorsalis* was the only species having its maximum abundance in August of both years, at the Rural site in 2006, and at the Rural and Sub/Rural sites in 2007.

Differences in seasonal abundance, and relative abundance of species among sites, may be influenced by weather and water usage patterns. January through September precipitation was near or below the 30-year mean in all months except August 2007, when 91.4 mm of rainfall was recorded. Of that amount, 55.6 mm (61%) fell on August 2. The increase in both *Culex* and *Aedes* abundance seen at the Natural, Rural, and Sub/Rural sites during August 2007, compared to August 2006, may have resulted, at least in part, from this rainfall event. The large spike in *Ae. dorsalis* abundance seen on August 24, 2006 at the Rural site was not associated with a rainfall event, so another factor, such as irrigation water usage, may have been responsible. Data on irrigation patterns were not available for analysis.

Although both years of the study had mean monthly temperatures at or above the 30-year norm during spring and early summer, the 2006 mosquito season began with temperatures above the norm from April to July, followed by temperatures at or below the norm in August and September. In contrast, in 2007 the April mean temperature was close to the 30-year norm, but temperatures were above the norm from May through September. Nevertheless, CDD accumulated in a similar curve in both years. Thus, temperature differences between the 2 years were probably not sufficiently large to account for the differences in seasonal abundance.

Host-seeking *Cx. tarsalis* were 1st detected in our study during the 1730–1930-h collection interval at the Natural and Sub/Rural sites, and at the other 2 sites during the 1930–2130-h collection interval. Host seeking by this species peaked at all 4 of our sites during the 2130–2330-h collection interval, beginning 1.2–2.7 h after sunset, then declined slowly throughout the night. Studies of *Cx. tarsalis* flight behavior in California depict a pattern of daily activities beginning with egress from diurnal resting sites, followed by sugar feeding, host seeking, and a return to resting sites near dawn (Reisen and Reeves 1990). In the Sacramento Valley of California, attraction of *Cx. tarsalis* to human bait peaked approximately 1 h after sunset, and continued at low levels throughout the night, with no early morning peak (Nelson and Spadoni 1972). Studies in Kern County, in the San Joaquin Valley, CA, using a time-segregated mosquito trap baited with CO_2 released at a constant rate, showed that host-seeking activity of both *Cx. tarsalis* and *Cx. quinquefasciatus* Say, the southern member of the *Cx. pipiens* complex, peaked within 1 or 2 h after sunset, with the *Cx. quinquefasciatus* peak occurring about 1 h earlier than that of *Cx. tarsalis* (Meyer et al. 1984). Another study using the time-segregated sampler compared the Kern Co. results to those obtained in the more arid Coachella Valley, CA (Reisen et al. 1997). Host seeking by *Cx. tarsalis* generally began at sunset, was most intense for 1–3 h, and then declined. Maximal host-seeking was observed during the hottest and driest time of the evening. Variations from this general pattern were attributed to distance from diurnal refugia, aerial adulticiding that reduced local vector populations, female abundance relative to bloodmeal host availability, and weather. Host-seeking activity peaked closer to sunset at the arid Coachella Valley site than at the Kern Co. site. We did not detect an early morning peak in *Cx. tarsalis* activity in our collections. Neither Reisen et al. (1997), nor the study of *Cx. tarsalis* attracted to human bait (Nelson and Spadoni 1972), detected increased host-seeking activity near sunrise, although Meyer et al. (1984) noted a small increase in activity at approximately 0400 h.

We were not able to analyze host-seeking activity patterns for *Cx. pipiens* because of the small number collected. This probably does not reflect the actual relative abundance of *Cx. pipiens* in the study area, but rather the lack of attractiveness for this species to CDC light traps placed near ground level. Studies conducted in the northeastern USA found that significantly more *Cx. pipiens* were collected in light traps placed in tree canopies than from traps placed near ground level (~1.5 m) (Anderson et al. 2004, Anderson et al. 2006; Andreadis and Armstrong 2007). In Memphis, TN, chicken-baited can traps were placed at 3 heights ranging from 3.1 to 7.6 m. Significantly more *Cx. pipiens* complex mosquitoes were collected in the highest trap than in the middle trap, but numbers collected in the lowest trap were not significantly different from numbers in the other trap heights (Savage et al. 2008). The authors suggest that this difference is due to *Cx. pipiens* questing for birds (their preferred bloodmeal host) that are nesting or roosting in the canopy. The use of this technique in northern Colorado may prove useful in collecting larger numbers of this species.

In our study, host-seeking by floodwater *Aedes* generally peaked earlier than *Cx. tarsalis*. Maximum numbers of *Aedes* were often collected during the 2130–2330-h interval, but this maximum was not significantly greater than the number collected during the 1930–2130 interval, beginning 0.8 h before to 0.7 h after sunset. This is in agreement with previous studies in California showing that *Ae. vexans* and *Ae. melanimon* activity peaked within 2 h after sunset (Nelson and Spadoni 1972; Meyer et al. 1986). However, Miura and Reed (1970) found that *Ae. melanimon* flight activity during summer followed a crepuscular pattern, with both evening and morning peaks of activity. In Ohio, Mitchell (1982) also found 2 peaks of *Ae. vexans* activity. The 1st peak occurred between 2100 and 2300 h, and the 2nd between 0400 and 0600 h, with reduced, but still substantial, activity between these peaks. In Connecticut, Anderson et al. (2007), using the same type of CBR as used in our study, found that *Ae. vexans* activity peaked in the 2 h following sunset, but did not decline significantly over the next 8 h. This is similar to the pattern we observed, although in our study trap counts dropped significantly from the maximum during the 0130–0330-h interval (6.5–7.5 h after sunset) at the Natural site, and by the 2330–0130-h interval (4.5–6.5 h after sunset) at the Rural and Sub/Rural site.

The consistency of our results across sites and years suggests that complicating factors mentioned above, such as distance of traps from daytime resting sites or host availability, were not critical factors affecting host-seeking activity. However, mosquito control activities may have affected abundance at the Natural and Sub/Rural sites, particularly the Urban site. During the 2003 WNV epidemic in Larimer County numerous *Cx. tarsalis* and *Cx. pipiens* were collected from the Urban site and the surrounding neighborhood, and 11 WNV-positive pools were detected in these 2 species (Nasci, unpublished data). Larviciding, and limited adulticiding, were initiated in Fort Collins in 2003, but only after epidemic activity was underway. Larviciding has continued in this area each year since 2003. During our 2006–2007 study, mosquito abundance was quite low at that site, and no WNV-positive pools were identified. Adulticides were not used during 2006, but ground-based ULV applications were done during 2007 on July 19–24 and August 28 (Schurich, personal communication) and likely affected mosquito abundance at the 3 sites mentioned above.

West Nile virus transmission patterns differed between study years, with 3 positive pools detected in 2006 vs. 11 in 2007. The Urban site was the only site where no WNV-positive pools were detected, as discussed above. Infected host-seeking females were collected during all time intervals except for the 1st and last intervals. Although IRs calculated with the use of aggregate data would not accurately represent WNV transmission intensity at different sites or dates, a comparison of the overall IR for each species may provide insight into its relative contribution to the transmission cycle. The highest IRs were found in *Cx. tarsalis* and *Cx. pipiens* (2.79 [95% CIs = 1.43–4.95] and 7.71 [95% CIs = 0.45–36.57], respectively), consistent with studies suggesting that these 2 species are important WNV vectors in northern Colorado (Bolling et al. 2007, Gujral et al. 2007). By contrast, the IR for *Ae. vexans* was 0.02 (95% CIs = 0.00–0.09), suggesting a minor role, at most, for this species in WNV transmission. The IR calculated for *Ae. melanimon* (IR = 0.21, 95% CIs = 0.04–0.70) was intermediate between that of *Ae. vexans* and the 2 *Culex* species. This is of interest as *Ae. melanimon*, typical of floodwater *Aedes*, feeds predominantly on mammals. Experimental studies have shown that several mammal species, such as eastern cottontail rabbits (*Sylvilagus floridanus*) (Tiawsirisup et al. 2005), chipmunks (*Tamias striatus*) (Platt et al. 2007), and fox squirrels (*Sciurus niger*) (Root et al. 2006, Platt et al. 2008), develop WNV titers sufficient to infect some mosquito species. Additionally, evidence of relatively high-titered natural WNV infections in fox squirrels in California have been reported (Padgett et al. 2007). In Larimer County serologic conversions were documented in fox squirrels naturally infected with WNV (Root et al. 2007). A secondary transmission cycle of WEEV between jackrabbits and *Ae. melanimon* in California has previously been described (Reisen 1984). It is possible, then, that an analogous cycle of WNV transmission involving 1 or more floodwater *Aedes* species and small mammals may occur.

A potential weakness of our study is the use of CO_2 produced by the sublimation of dry ice as bait, rather than a steady flow rate of CO_2 gas from a cylinder. Laboratory studies of CO_2 sublimation rates from 1 kg of dry ice in an insulated container held at 27°C ambient temperature found that the rate decreased from ~1.0 liter/min to 0.425 liter/min over a 12-h period (Pfuntner et al. 1988). Reisen et al. (2000) compared different methods of CO_2 presentation on *Cx. tarsalis* abundance in traps with the use of dry ice, or gas from cylinders at flow rates of 0.5, 1.0, and 1.5 liters/min, rates similar to the amounts produced by sublimated dry ice. Traps baited with gas from cylinders caught significantly more *Cx. tarsalis* than dry-ice–baited traps, although the authors were unable to explain this, given that *Cx. tarsalis* at their study sites were active primarily before midnight (Reisen et al. 1997) when dry-ice sublimation rates would still be relatively high. Given that our traps were set out between ~1600 and 1700 h, and collected increasing numbers of *Cx. tarsalis* and floodwater *Aedes* until sometime between 2130 and 0130 h, we feel that our results accurately represent the host-seeking activity of these species. Supporting this, a study in Connecticut using CBR found that host-seeking activity patterns of *Cx. pipiens*, *Cx. salinarius* Coq., and *Ae. vexans* collected in dry-ice–baited traps in 2004–2005 were very similar to the patterns seen in 2006 with CO_2 released at a standard flow rate throughout the night from tanks (Anderson et al. 2007). However, as we collected traps 30 min or more after the end of the last collection interval, we cannot confirm that dry ice still remained in the traps during that interval. Thus, it is possible that we would not have detected a 2nd peak at sunrise, if one existed.

The use of CO_2-baited traps might add a confounding variable that should be considered in monitoring mosquito flight activity. Data presented in the above studies of host-seeking behavior were visually examined. With the exception of 1 study (Reisen et al. 1997, which includes results from Meyer et al. 1984), host-seeking activity was detected at higher levels later into the night in studies utilizing CO_2-baited traps (Mitchell 1982, Anderson et al. 2007, our study) than in studies using landing counts (Nelson and Spadoni 1972) or unbaited traps (Miura and Reed 1970). If this observation is valid, a possible explanation is that the constant expression of CO_2 during the night, especially when the weather is calm, stimulates host-seeking behavior in mosquitoes in the vicinity of the trap that otherwise might have ceased flight. Supporting this, Reisen et al. (2000) found that traps releasing CO_2 in pulses, to simulate host exhalations, collected fewer mosquitoes than did traps baited with dry ice or with CO_2 released at a constant rate. However, the authors argue that changing wind patterns during the night, and landscape features, would disrupt the effects of constant CO_2 release. Also, Meyer et al. (1986), using CO_2 released at a constant rate, found that *Cx. tarsalis* host-seeking extended longer into the night at the site where CO_2 abundance was greatest. They attributed the longer duration of host-seeking to density-dependent success in obtaining a bloodmeal; either low host density or high mosquito density stimulating increased avoidance behavior by available hosts. If constant CO_2 release does modify host-seeking activity it is probably in the direction of extending the length of host-seeking rather than by skewing the peak time of activity.

Our findings have implications for mosquito surveillance and control activities in northern Colorado and the Great Plains region. First, relative abundance and seasonality were highly variable among sites and between years. This argues for the utility of a standardized mosquito trapping plan that begins early enough in the season to allow recognition of variations in seasonal abundance patterns, and to allow for control efforts to be focused optimally. Second, regarding the timing of adulticide applications, our results suggest that optimal control of *Cx. tarsalis* should be initiated at least 1 h after sunset. Applications to control the floodwater *Aedes* should begin at or soon after sunset, when atmospheric stability is optimal for aerosol application of adulticides (Mount 1998) and human outdoor activity is reduced. As the numbers of females of all species collected declined slowly throughout the night in most cases, subject to the caveats discussed above, adulticide applications could continue throughout most of the night.

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