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Ultra Low Volume (ULV) Adulticide Spraying in Response to Host-seeking and Oviposition Activity of Mosquitoes (Diptera: Culicidae) Associated with West Nile Virus and Eastern Equine Encephalitis Transmission in Massachusetts, U.S.A.

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Abstract

Mosquito host-seeking and oviposition activity was studied to determine: (1) an optimal ultra low volume (ULV) insecticide spraying time, and (2) if more than one application would be needed to target potential West Nile virus (WNv) and Eastern Equine Encephalitis (EEE) virus mosquito vectors. A collection bottle rotator trap was used to collect host-seeking mosquitoes, whereas a modified bottle rotator oviposition trap was used to collect gravid mosquitoes in five 3-h intervals: one period before sunset (-3 to 0 h), one at sunset (0-3 h), and three thereafter (3-6 h, 6-9 h, 9-12 h). All mosquito species host-seeking activity combined showed peak activity at the 0-3 h interval relative to sunset. *Culex* spp. (*Culex pipiens* (Linnaeus) and *Cx. restuans* (Theobald)) oviposition activity was significantly highest at the 0-3 h interval relative to sunset, declined at 3-6 h, and then significantly increased to a steady level thereafter. The data suggest that: (1) the 0-3 h interval relative to sunset as the optimal time for ULV insecticide spraying for vector control and that (2) only one ULV application is necessary to effectively control host-seeking and ovipositing mosquito populations.

Key words

ULV, WNv, EEE, oviposition, host-seeking

Introduction

Eastern Equine Encephalitis (EEE) is a mosquito-borne disease caused by an alphavirus of the *Togaviridae* family of viruses. It was first discovered in Massachusetts in 1938 with 34 cases reported, of which 25 resulted in fatalities (Fothergill et al. 1938, Getting et al. 1941). EEE virus is maintained in an enzootic cycle between mosquito vectors and avian reservoirs, which serve to amplify the virus. In the United States, an average of five to seven human EEE neuroinvasive viral cases is reported annually. A total of 260 human cases has been reported between 1964 and 2009, with a mortality rate of about one third (CDC 2011a). Survivors often experience long term mental and physical sequelae (CDC 2011a, Przelomski et al. 1988).

Massachusetts is one of the most active EEE foci in the United States, second only to Florida (~ 14% and ~ 25% of all cases reported, respectively; CDC 2011a). Historically, Plymouth and Bristol Counties tend to be the foci of EEE virus activity within Massachusetts (MDPH 2011ab, Moncayo and Edman 1999). The most recent outbreaks occurred in 2006 and 2010. In 2006, 157 of 9,344 mosquito pools tested positive for EEE and five human cases, with two resulting in fatalities, were identified. In 2010, a similar outbreak occurred after 65 of 3,558 mosquito pools tested positive for EEE and two human cases were indentified. In both scenarios, the *Culiseta melanura* (Coquillett) mosquito species had the highest incidence of EEE positive pools, followed by *Coquillettidia perturbans* (Walker) (MDPH 2011ab).

Appearing in New York City in 1999, West Nile virus (WNv) is relatively new to the United States when compared to EEE virus (CDC 2011b, Hayes 2005). As with EEE, WNv is maintained in the enzootic cycle between mosquito vectors and avian reservoirs. Unlike EEE, West Nile is a flavivirus of the *Flaviviridae* family. Since the appearance of WNv in the United

States, there has been an average of 2,555 reported cases (neuroinvasive, non-neuroinvasive, and unspecified) and an average death toll of 101 annually (CDC 2011b; Table 1).

Comparing WNv incidences to the EEE outbreaks in Massachusetts in 2006, 43 of 9,344 mosquito pools tested positive with three human cases reported. In 2010, 121 of 3,558 mosquito pools tested positive for WNv, resulting in seven human cases (MDPH 2011ab). In these cases, *Culex* spp. (*Cx. pipiens* (Linnaeus) and *Cx. restuans* (Theobald)) was the culprit vector, followed by *Cs. melanura*, for the spread of West Nile virus.

Combining all WNv and EEE cases in Massachusetts, 2.1% of all tested mosquito pools were positive for virus in 2006, and that figure more than doubled to 5.2% for 2010. Source reduction, such as eliminating breeding habitat, is the most environmentally sensitive control method as part of an Integrated Pest Management (IPM) program (Axtell 1979, Olson 1979, Rose 2001), better referred to as Integrated Mosquito Management (IMM) by the American Mosquito Control Association (2011). Inevitably there will be adult mosquitoes, and when they pose a public health risk, immediate control measures are advised to reduce the spread of virus. Contemporary ultra low volume (ULV) applications employ very low amounts of insecticide to reduce potential disease-vectoring mosquito populations and can be applied either by specialized trucks or airplanes.

To reduce potential environmental effects and to improve control efficacy, it is imperative that ULV programs operate when target vector mosquitoes are most active. Our research studies the host-seeking and oviposition activity of mosquitoes in Massachusetts and explores: (1) an optimal ULV insecticide spraying time, and (2) if more than one application would be needed to target potential West Nile virus and/or Eastern Equine Encephalitis virus mosquito vectors.

Materials and methods

Trap designs

Host-seeking trap. A John W. Hock Collection Bottle Rotator (Model 1512, Gainesville, FL) was mounted onto a wooden post approximately 5 ft in length (~1.5 m) staked into the ground (Fig. 1). A hose connected to a carbon dioxide tank was attached to a CDC miniature light trap mounted on top of the bottle rotator unit.

Oviposition trap. A John W. Hock Collection Bottle Rotator (Model 1512, Gainesville, FL) was mounted on a wooden base high enough to allow rotation of the collection bottles without contacting the ground. Various 3 in diameter (~8 cm) PVC piping was fashioned and connected from the intake of the bottle rotator unit to a plastic tub baited with infused hay solution (Fig. 2). A fan unit (without light bulb) from a CDC miniature light trap was attached at the tub-end of the PVC piping with a reducer to mitigate turbulence on the water surface. The PVC piping was supported over the tub by means of a pipe hanger attached to a piece of wood the width of the tub. All piping connections were secured with PVC primer and glue.

Host-seeking activity

Host-seeking traps were set in woody, shaded areas typical of mosquito habitat in sites across Norfolk and Worcester Counties, Massachusetts from 5^{th} June through 8^{th} September, 2008 and 9^{th} June through 15^{th} September, 2009 for a total of 130 trap nights. Carbon dioxide attractant from a tank was output at 250-500 cc. The rotator units were programmed for five 3-h collection intervals: one period before sunset (-3 to 0 h), one at sunset (0-3 h), and three thereafter (3-6 h, 6-9 h, 9-12 h). Trap intervals were reprogrammed approximately every two weeks to adjust around sunset. After every trapping session, mosquitoes were collected and stored frozen for later identification using morphological characteristics (Andreadis et al. 2005).

Oviposition activity

The modified bottle rotator oviposition traps were set in woody, shaded areas at two sites in Norfolk County and at three sites in Worcester County, Massachusetts from 28th June through 22nd September, 2010 for a total of 52 trap nights. The trap pans were filled with hay-infused water as an attractant to about 2-3 in (~5-7 cm) from the fan intake. The rotator units were programmed for five 3-h collection intervals: one period before sunset (-3 to 0 h), one at sunset (0-3 h), and three thereafter (3-6 h, 6-9 h, 9-12 h). Trap intervals were reprogrammed approximately every two weeks to adjust around sunset. After every trapping session, mosquitoes were collected and stored frozen for later identification using morphological characteristics.

Statistical analysis

Data were $\log_e$ transformed with zero counts replaced with 1's for geometric means comparisons (Microsoft Office Excel 2007, Microsoft Corporation, Redmond, WA). Geometric means for collection intervals were compared with the Kolmogorov-Smirnov two-tailed independent samples test (K-S test) using statistical analysis software (SPSS 16, IBM Corporation, Somers, NY). Trap nights with no collections of a particular species were not considered in the analysis for that species.

Results

Host-seeking activity

Seven species of mosquitoes were collected from 2009 through 2010 (Table 2), and, except for *Anopheles* spp. (*Anopheles punctipennis* (Say) and *An. quadrimaculatus* (Say)) and *Psorophora ferox* (von Humboldt), were analyzed with the aforementioned K-S tests. *Culex* spp. (*Culex pipiens* (Linnaeus) and *Cx. restuans* (Theobald)) host-seeking activity was significantly highest at sunset (0-3 h; $n = 79$, $P < 0.05$), but declined and remained steady as the evening progressed (Fig. 3A). *Culiseta melanura* (Coquillett) and *Coquillettidia perturbans* (Walker) host-seeking activity was also significantly highest at the sunset interval (0-3 h; $n = 119$ and $n = 130$, $P < 0.05$, respectively), whereas activity within the other intervals was equal to one another (Figs. 3B and 3C). *Aedes vexans* (Meigen) and *Ochlerotatus canadensis* (Theobald) host-seeking activity did not differ significantly through the trapping intervals (Fig. 3D, $n = 94$ and Fig. 3E, $n = 71$, $P > 0.05$, respectively). All mosquito species host-seeking activity combined showed peak activity at the 0-3 h interval relative to sunset (Fig. 3F, $n = 563$, $P < 0.05$).

Oviposition activity

A total of 2,531 gravid *Culex* spp. mosquitoes was collected from the modified bottle rotator oviposition trap. *Culex* spp. oviposition activity was significantly highest at the sunset interval (0-3 h, $n = 52$, $P < 0.05$), declined at 3-6 h, then increased significantly once more for the remainder of the evening (Fig. 4).

Discussion

We chose to analyze host-seeking and oviposition activity of specific mosquito species based on previous studies implicating their roles as either enzootic and/or bridge vectors in the West Nile virus or Eastern Equine Encephalitis cycles. For our purposes, we consider *Culex* spp. as a complex of *Cx. pipiens* and *Cx. restuans* mosquitoes, which is how they are identified and labeled by the Massachusetts Department of Public Health and Massachusetts mosquito control organizations. *Culex* spp. has been heavily scrutinized as one of the most important enzootic vectors of WNv (Savage et al. 2006, 2008, Apperson et al. 2002, 2004), although recent analysis by Kilpatrick et al. implicates the *Culex* spp. complex as bridge vectors and that they may be responsible for up to 80% of human WNv infections (2005). We observed *Culex* spp. host-seeking activity to be most significant at the sunset interval (0-3 h), with the intervals after still quite active (Fig. 3A). While this behavior has also been noted in previous studies (Anderson et al. 2007, Crisp and Knepper 2002, Mitchell 1982, Reddy et al. 2007, Savage et al. 2008, Suom et al. 2010), Gladney and Turner (1970) have observed only an even distribution of activity of *Cx. restuans* mosquitoes throughout the night.

Culiseta melanura has long been considered an enzootic vector of EEE (Hayes 1962, Molaei et al. 2006). In a more recent finding, weekly EEE virus minimal infection rates (MIR, defined as the number of virus positive pools per 1,000 mosquitoes tested (Moore et al. 1993)) per trap night strongly associate *Cs. melanura* with human infection in spite of their population decline since 1979 (Hachiya et al. 2007). Even though *Cs. melanura* is mostly ornithophilic, Molaei et al. (2006) observed that more than 10% of their blood-meals were mammalian-derived, implicating it as a bridge vector of WNv to mammals. Our observation of *Cs. melanura* host-seeking activity peaking at sunset (Fig. 3B) agrees with previous studies (Crans et al. 1982, Hayes 1962, Nasci and Edman 1981).

Analyses by Moncayo and Edman suggest *Coquillettidia perturbans* as an epidemic vector of EEE virus in southeastern Massachusetts through rankings based on populations of mosquitoes caught in CO_2-baited light traps (1999). The role of *Cq. perturbans* as a bridge vector of EEE and WNv has also been suggested by Apperson et al. (2002) as well as by Molaei et al. (2008) because of their preference for mammalian blood and opportunistic feeding of avian blood. We observed *Cq. perturbans* host-seeking activity peak 0-3 h after sunset (Fig. 3C), which corroborates findings of previous studies (Anderson et al. 2007, Crisp and Knepper 2002, Gladney and Turner 1970, Hayes 1962, Taylor et al. 1979).

Aedes vexans should also be considered an important bridge vector because of its high population densities, repeated detection of WNv in the species, and its preference for mammalian blood (Turell et al. 2005). We observed *Ae. vexans* host-seeking activity as relatively even throughout the night (Fig. 3D). Others have noted a peak shortly after sunset (Anderson et al. 2007, Mitchell 1982, Wright and Knight 1966), whereas Gladney et al. (1970), Nelson and Spadoni (1972), Crisp and Knepper (2002), and Aldemir et al. (2010) note peaks at dusk and in the early morning periods. The differences observed in *Ae. vexans* behavior may be a matter of geography, reflecting the local variance of the species. Alternatively, our data may not be representative since we observed lower than average *Ae. vexans* populations for the last two years in Massachusetts.

The role of *Ochlerotatus canadensis* as a bridge vector of EEE virus has been suggested by Moncayo and Edman (1999) and Molaei et al. (2008); but in the case of WNv, observations under laboratory conditions have described them as inefficient vectors (Turell et al. 2005). Our data suggest no significant differences in host-seeking activity intervals during the trapping period (Fig. 3E). Mitchell (1982) and Crisp and Knepper (2002) report of higher activity before sunset whereas Hayes (1962) reports diurnal activity with crepuscular peaks.

Grouping all mosquito species host-seeking activity together for analysis indicates the 0-3 h period around sunset as significantly more active than all other intervals (Fig. 3F). Our data and analysis of general mosquito host-seeking activity agrees with previous studies (Carroll and Bourg 1977, Savage et al. 2008, Suom et al. 2010) of mosquito questing behavior.

Oviposition activity of mosquitoes has not been fully considered in the implementation of ULV insecticide spraying programs since most abundance data are collected from CDC miniature light traps. We sought to improve the efficacy of such programs by studying the temporal oviposition activity of gravid *Culex* spp. mosquitoes. Although Reddy et al. did not observe bi-modal peaks in *Culex* spp. oviposition activity (2007), our findings indicate peaks at both sunset and towards sunrise (Fig. 4), corroborating a recent study by Savage et al. (2006) and an earlier one by MacDonald et al. (1981). When comparing *Culex* spp. host-seeking to oviposition activity (Fig. 5), peak activities coincide at the sunset interval (0-3 h). Another note is that average *Culex* spp. oviposition activity is significantly higher than average host-seeking activity at sunset (0-3 h interval) and in the early morning hours (6-9 h, 9-12 h intervals), reinforcing the importance of gravid trap usage for vector surveillance. When *Culex* spp. oviposition activity is compared to all mosquito species host-seeking activity combined, the peak activity interval also coincides (Fig. 6) at the sunset interval (0-3 h), with *Culex* spp. oviposition activity significantly higher from sunset until morning. Having observed peak mosquito host-seeking and oviposition activity as simultaneous events (Fig. 6), there is no need for more than one ULV application to treat an area.

Research studies have generally agreed that mosquito spraying efforts initiated after sunset would coincide with the highest flight activity of the evening (Anderson et al. 2007, Carroll and Bourg 1977, Mitchell 1982, Reddy et al. 2007, Savage et al. 2006, Suom et al. 2010). Anderson et al. (2007) and Carroll and Bourg (1977) suggest mosquito ULV spraying efforts be conducted from sunset to sunrise for the greatest application efficacy, although Crisp et al. (2002) would include at least 1-2 h before sunset in regards to spring mosquito species. For public health operations and logistical purposes, our research hopes to narrow the application time interval for greatest efficacy for control of disease-vectoring mosquitoes. Kilpatrick et al. (2005) recommend focusing on control of *Cx. pipiens* and *Cx. restuans* since they may be responsible for up to 80% of human WNv infections in the northeastern United States. Therefore, targeting older, blood-fed and ovipositing female mosquitoes would be beneficial since they are more likely to be infected, thus a 2-h evening and a 2-h morning application is recommended by Savage et al. (2006). On the other hand, our data suggest that the optimal time to apply ULV insecticide to target WNv and EEE vectors would be 0-3 h after sunset. This would eliminate the need for multiple applications and excess chemical usage since peak mosquito host-seeking and oviposition activities occur simultaneously (Figs. 3F, 4, and 6). Even still, mosquito adulticide applications conducted thereafter are warranted since: (1) *Culex* spp. oviposition activity is rather significant towards the early morning hours (Fig. 4), and (2) implicated bridge vector species activity is equal throughout the evening (Figs. 3D and 3E). Mosquito management practices will vary by region and are dictated by local ordinances, needs, and differences in behavior of mosquito sub-species in geographically different areas. The research and data presented here were accomplished for the matter of vector control logistics and should be used merely as a reference.

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Tables

Table 1. West Nile virus cases reported to the Centers for Disease Control and Prevention, 1999-2010.

Year	Neuroinvasive Disease Cases	Non-neuroinvasive Deisease Cases	Unspecified Disease Cases	Total	Fatalities
1999	59	3	0	62	7
2000	19	2	0	21	2
2001	64	2	0	66	10
2002	2,946	1,160	50	4,156	284
2003	2,866	6,830	166	9,862	264
2004	1,142	1,269	128	2,539	100
2005	1,294	1,607	99	3,000	119
2006	1,459	2,616	194	4,269	177
2007	1,217	2,350	63	3,630	124
2008	687	624	45	1,356	44
2009	373	322	25	720	32
2010	601	380	0	981	45
Total	12,727	17,165	770	30,662	1,208
Average	1,061	1,430	64	2,555	101

Table 2. Host-seeking mosquitoes collected by species and year. *Anopheles* spp. consist of *Anopheles punctipennis* and *An. quadrimaculatus*.

	Year		
Species	2008	2009	2008-09 combined
Culex spp.	1,057	363	1,420
Culiseta melanura	292	700	992
Coquillettidia perturbans	14,837	23,689	38,526
Aedes vexans	239	632	871
Ochlerotatus canadensis	-	740	740
Anopheles spp.	506	930	1,436
Psorophora ferox	-	582	582
Total	16,931	27,636	44,567

Figures

Figure 1. Collection bottle rotator trap

Figure 2. Modified bottle rotator oviposition trap

Figure 3. Mosquito host-seeking activity relative to sunset. Geometric means for each interval with the same lowercase letter are not significantly different ($P < 0.05$; K-S test).

Figure 3A. *Culex* spp. (n = 79)

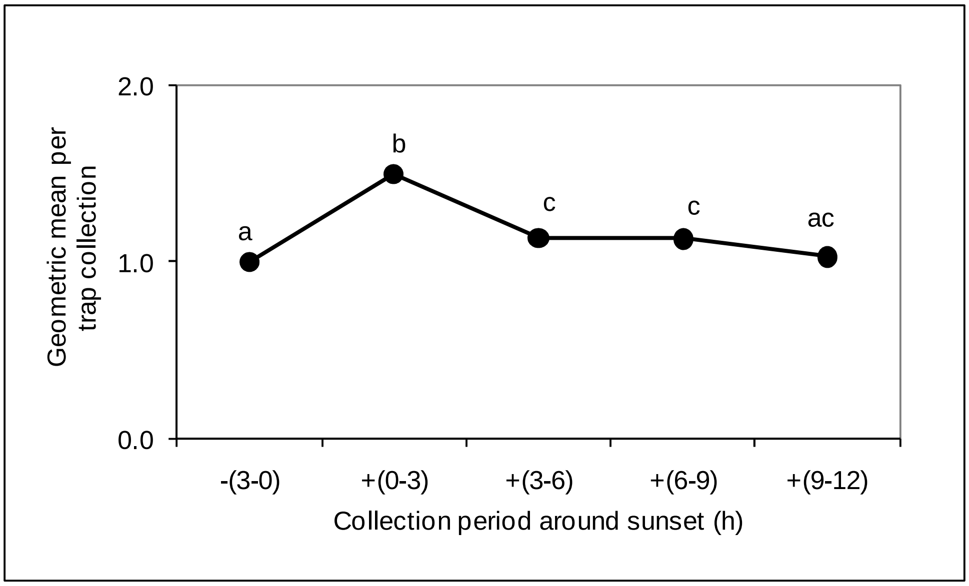

Figure 3B. *Culiseta melanura* (n = 119)

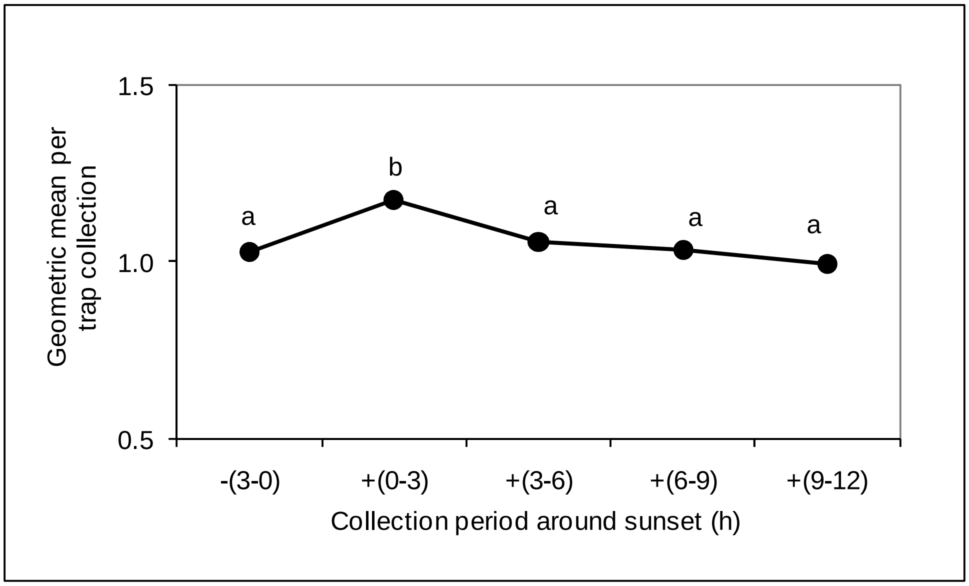

Figure 3C. *Coquillettidia perturbans* (n = 130)

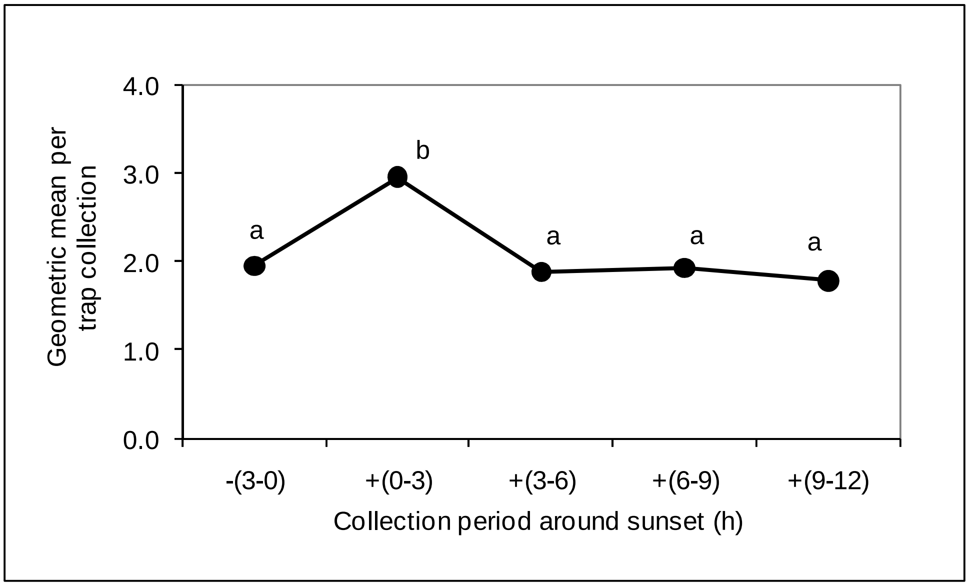

Figure 3D. *Aedes vexans* (n = 94)

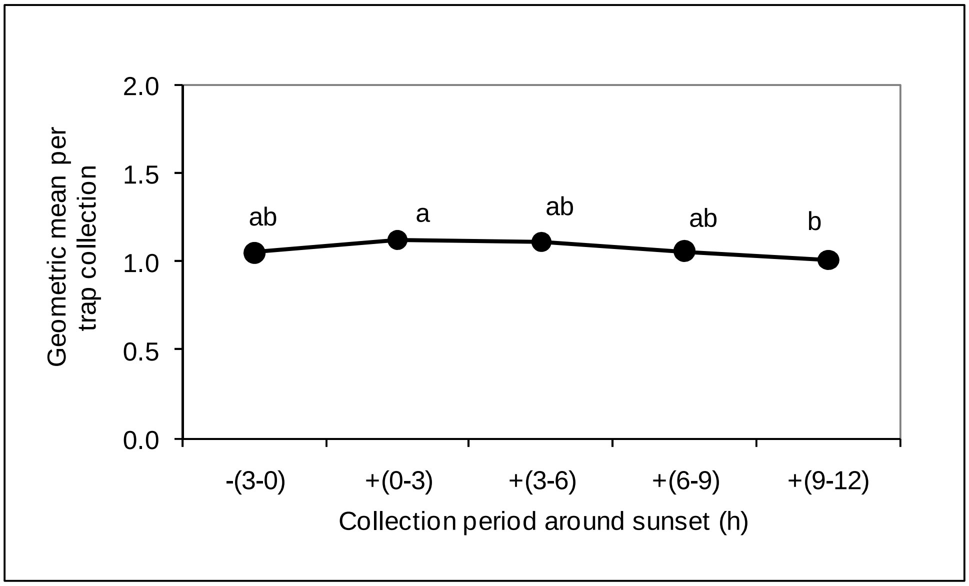

Figure 3E. *Ochlerotatus canadensis* (n = 71)

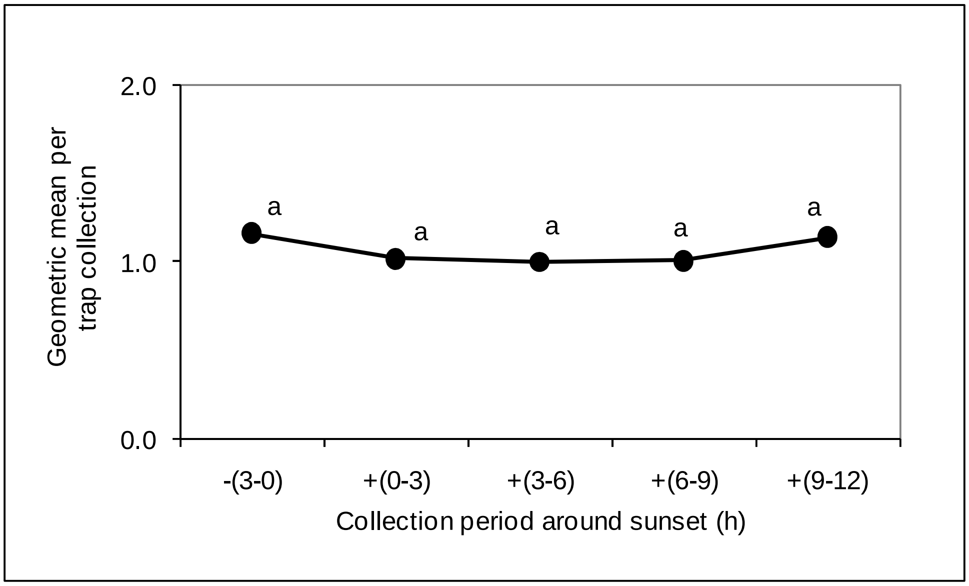

Figure 3F. All mosquito species combined (*n* = 563)

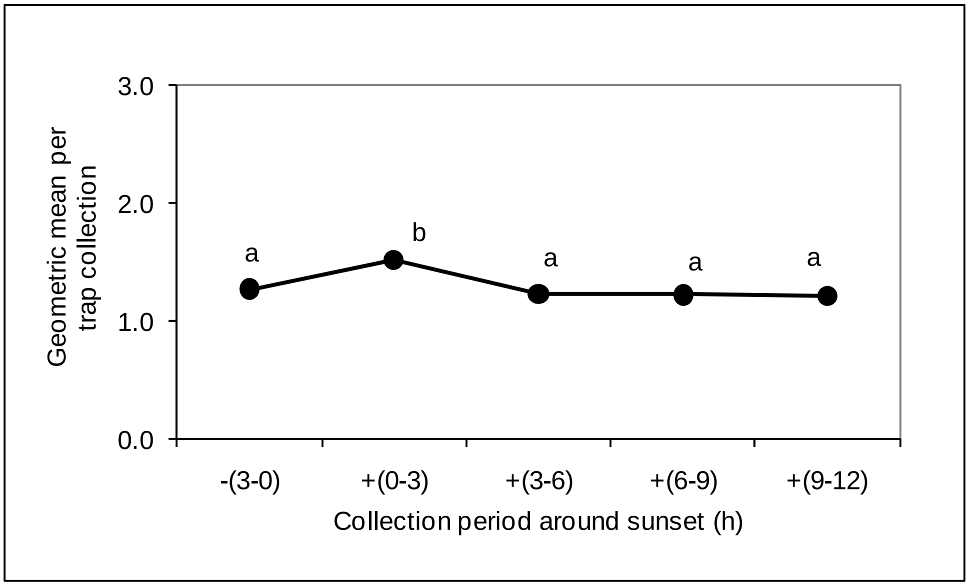

Figure 4. *Culex* spp. oviposition activity. Geometric means for each interval with the same lowercase letter are not significantly different (K-S test, n = 52, $P < 0.05$).

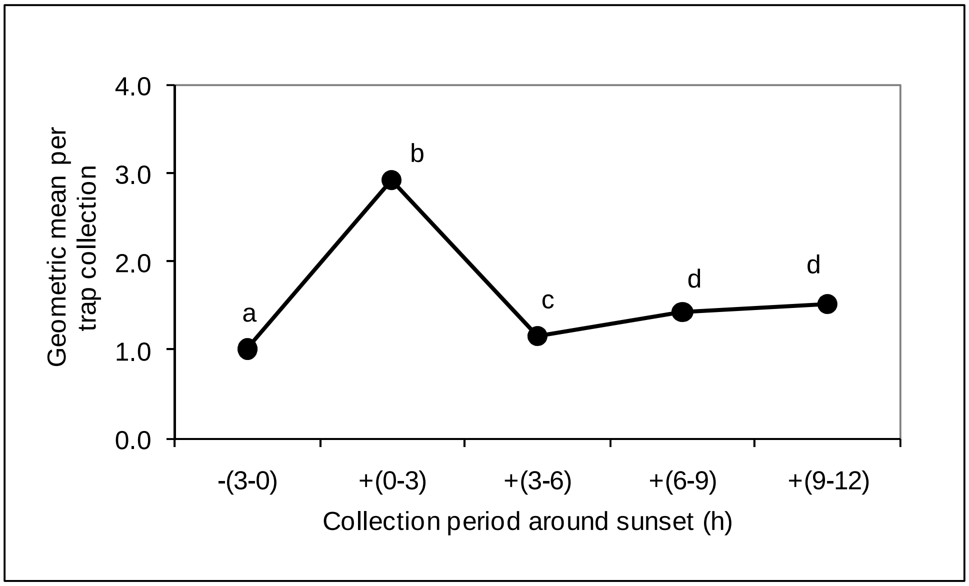

Figure 5. *Culex* spp. host-seeking activity (n = 79) compared to oviposition activity (n = 52). Time intervals with an asterisk (*) indicate a significant difference (K-S test, $P < 0.05$).

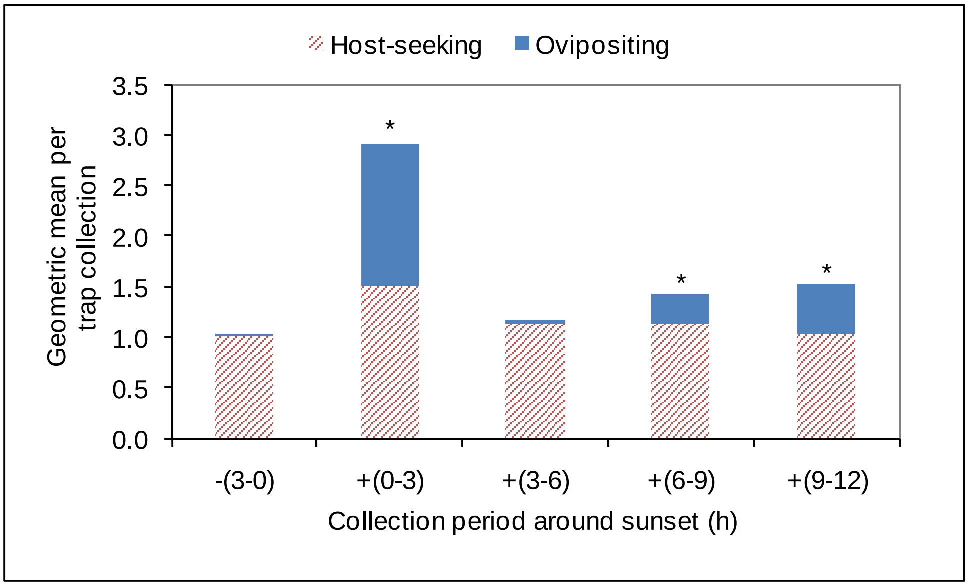

Figure 6. All species combined host-seeking activity (n = 573) compared to *Culex* spp. oviposition activity (n = 52). Time intervals with an asterisk (*) indicate a significant difference (K-S test, P < 0.05).