# Mosquito Control Program Vector Surveillance Submission Guidance
## *Summer 2024*

### Executive Summary

The purpose of this document is to provide guidance for county mosquito control agencies (CMCAs) to help target state supported surveillance efforts on vectors of concern to prevent human illness. Recommendations for additional mosquito surveillance to support identified research priorities will be provided separately as needed. While this document lists key species to target for each pathogen of interest, there are other species that CMCAs may also want to consider as part of their surveillance program. Decisions on which species to target and the number of pools/pathogen to submit should be based on local mosquito populations and local/regional arboviral activity. The State Mosquito Control Commission (SMCC) through the Office of Mosquito Control Coordination (OMCC) and/or the New Jersey Agricultural Experiment Station (NJAES) through the Rutgers Center for Vector Biology (CVB) will consult with CMCAs on emerging surveillance needs and strategies.

**Mosquito-Based Surveillance**

Mosquito-based surveillance consists of the systematic collection and identification of mosquito samples and screening them for arboviruses. The principal functions of a mosquito-based surveillance program are to:

- Collect data on mosquito population abundance and virus infection rates in those populations.
- Provide indicators of the threat of human infection and disease and identify geographic areas of high-risk.
- Support decisions regarding the need for and timing of intervention activities (i.e., enhanced vector surveillance and control, use of new technologies, and public education programs).
- Monitor the effectiveness of vector control efforts, including susceptibility of target mosquitoes to control methods used.

**Arboviruses of Concern**

In New Jersey, West Nile virus (WNV) is the most common mosquito-borne disease with an annual endemic average of 7 human cases and periodic epidemic years with 20-61 human cases. Eastern equine encephalitis (EEE) is also detected annually in mosquito pools and horses, but human cases to date have been rare. The New Jersey Department of Health (NJDOH) started testing for Jamestown Canyon (JCV) in 2019, which has resulted in a low number of detections to date; only four human cases of this emerging infection have been reported in N.J. Although more common in states to the south of N.J., human cases of La Crosse virus (LAC) have been reported in the past in N.J. and have been detected in mosquitoes in recent years. Saint Louis encephalitis (SLE) is a mosquito-borne virus disease that was widely distributed in the continental U.S. Large outbreaks in N.J. occurred in 1964 and 1975 with activity documented in Burlington, Camden, Middlesex, and Monmouth counties. Fortunately, this virus has not been a major concern in recent years, but sporadic human cases are reported in Pennsylvania. After the global Zika pandemic in 2016, awareness of the need to prevent travel-associated arboviruses [Chikungunya (CHIK), Dengue (DENV), Zika (ZIKV)] from entering local mosquito populations has increased and requires different mosquito surveillance and control strategies.

**Malaria**

Surveillance and control of mosquitoes that can transmit malaria has focused on nuisance concerns and for arboviruses but haven't been specifically addressed to prevent malaria in N.J. since the mid-20$^{th}$ Century. However, the sporadic detection of locally acquired cases in the United States in recent years, along with a high number of travel-associated human malaria cases reported in N.J. has demonstrated the need for continued vigilance.

**Trapping**

When selecting a mosquito trap for disease surveillance, it should be noted that host-seeking traps including $CO_2$-baited light traps, New Jersey Light Traps (NJLT), CDC miniature light traps (CDC), Biogents – Sentinel traps (BGS), etc. introduce a bias and will collect mostly (65-80%) unfed nulliparous females, resulting in a large proportion of the mosquitoes sampled testing negative. Traps that target gravid females (mosquitoes looking to lay eggs), will collect a much larger proportion of mosquitoes that have taken a blood-meal, increasing the likelihood of viral exposure obtained through these blood feeding behaviors.

**Arboviral Testing**

Arboviral testing in mosquito pools is performed at two public health laboratories, the N.J. Public Health and Environmental Laboratories (PHEL) and the Cape May County Department of Mosquito Control Bio-safety Level 3 Laboratory. There are three multiplex testing options available for CMCAs when submitting mosquito pools:

1. WNV Panel (WNV, EEE, SLE, JCV)
2. La Crosse Panel (WNV, LAC)
3. Exotic Aedes Panel (CHIK, DENV, ZIKV)

Owing to the use of multiplex testing panels, species may be tested for pathogens other than the ones that are solicited. As a result, species may test positive for pathogens that aren't thought to be directly involved in transmission, e.g., *Cx. pipiens* that test positive for EEE. CMCAs are asked to sample from areas of local concern and submit 15-20 mosquito pools per week during the active mosquito surveillance season. NJ's vector control season has expanded, consistent with a longer warm weather climate. Prior to 2021, due primarily to fiscal constraints, arbovirus surveillance efforts started at the beginning of June. This decision was adopted following several years of data that established clear WNV seasonal activity trends. In response to earlier virus detections, however, starting in 2021 vector surveillance now starts in mid-May, to better characterize recent early season activity. The mosquito surveillance season end date is variable and is based on environmental factors, mosquito abundance, and arboviral pathogen activity, but is usually between late-October and mid-November.

***Plasmodium* spp, Testing**

Testing mosquitoes for *Plasmodium* spp., the parasite that causes malaria, is not routinely recommended, and is not performed in N.J. If mosquito testing for *Plasmodium* spp. is warranted, it would be performed at the Centers for Disease Control and Prevention (CDC).

**Summary of Surveillance by Human Pathogen**

WNV & SLE Surveillance:

- Targeted species: *Cx. pipiens, Cx. restuans, Cx. salinarius, Ae. taeniorhynchus, An. quadrimaculatus*
- Timing: Throughout surveillance season
- Geography: All N.J. counties
- Traps: Gravid, $CO_2$ -baited light traps, BGS
- Volume: 10-15 pools/week
- Testing: WNV Panel

EEE Surveillance:

- Targeted species: *Cs. melanura, Cq. perturbans, Cx. erraticus*
- Timing: Throughout surveillance season
- Geography: All N.J. counties
- Traps: Resting boxes with un-baited light trap(s) (preferred), gravid traps
- Volume: 2-5 pools/week (apart from state resting box sites)
- Testing: WNV Panel

JCV Surveillance:

- Targeted species: Early season *Aedes* species and others as described in JCV section
- Timing: Late April/early May and throughout the season
- Geography: All N.J. counties
- Traps: $CO_2$-baited light traps
- Volume: 2-4 pools/week
- Testing: WNV Panel

LAC Surveillance:

- Targeted species: *Ae. triseriatus*
- Timing: Late April/early May through end of surveillance season
- Geography: All N.J. Counties
- Traps: $CO_2$-baited BGS, $CO_2$- baited light traps (set low to the ground)
- Volume: 1-2 pools/week
- Testing: LAC Panel

Exotic Aedes Surveillance Summary:

- Targeted species: *Ae. albopictus, Ae. aegypti*
- Timing: Throughout surveillance season, and/or in response to human cases
- Geography: Statewide surveillance; focused testing only
- Traps: BGS
- Surveillance: 1-2 traps/week

- Testing Volume: No minimum; in response to human case notifications, or local needs
- Testing: Exotic Aedes Panel

Malaria Surveillance Summary:

- Targeted species: *Anopheles quadrimaculatus, An. punctipennis*, *An, crucians*, *An. bradleyi*
- Timing: In response to suspected locally-acquired human cases
- Geography: Focused testing only
- Traps: Resting Box, CDC, gravid
- Surveillance: As indicated, in response to suspected locally-acquired human cases
- Testing Volume: No minimum; in response to suspected locally-acquired human cases
- Testing: To be determined at CDC

## Vector Surveillance Guidance by Pathogen of Concern

**West Nile & St. Louis Encephalitis Virus Surveillance**

Human WNV disease cases have been reported during every month of the year in the United States, but as is characteristic of zoonotic arboviruses in temperate climates, intense transmission is limited to the summer and early fall months. Ninety-four percent of human cases have been reported from July through September, and approximately two-thirds of reported cases occur during a 6-week period from mid-July through the end of August. Weather, especially temperature, is an important modifier of WNV transmission and has been correlated with increased incidence of human disease at regional and national levels. Weather likely drives the annual fluctuations in numbers of cases reported at the national level. In N.J., WNV is the most common mosquito-borne disease with an annual endemic average of 7 human cases and periodic epidemic years with 20-61 human cases.

WNV is primarily maintained in an enzootic transmission cycle between *Culex* species mosquitoes and birds as the vertebrate hosts. Some infected birds can develop high levels of the virus in their bloodstream and mosquitoes become infected by biting infected birds. After about a week, infected mosquitoes can pass the virus to other birds, people, horses, and other mammals. Humans, horses, and other mammals are "dead end" hosts, meaning they do not develop sufficiently high levels of virus in their bloodstream to transmit the virus to other biting mosquitoes. By monitoring WNV infection prevalence in mosquito vectors and incidence in non-human vertebrate hosts and comparing these indices to historical environmental and epidemiological surveillance data, conditions associated with increasing human risk can be detected 2-4 weeks in advance of human disease onset. This provides additional lead time for critical vector control interventions and public education programs to be put in place.

St. Louis encephalitis (SLE) is maintained in a similar bird-mosquito cycle as WNV and shares the same primary vectors. Most cases historically have occurred in eastern and central states, where episodic urban-centered outbreaks have recurred since the 1930s. However, in more recent years, sporadic cases and outbreaks have occurred in the Southwest. In temperate areas of the United States, SLE cases occur primarily in the late summer or early fall. N.J. last reported a human case of SLE in 1978. According to CDC records, N.J. has had a total of 131 cases with the majority occurring in 1964 and 1975.

<u>WNV & SLE mosquito species of concern</u>

The principal mosquito vectors for WNV and SLE on the East Coast are members of the genus *Culex (Cx)*, primarily *Cx. pipiens* and *Cx. restuans*. These species may be abundant in urban areas inhabiting artificial containers (e.g., birdbaths, discarded tires, buckets, clogged gutters) as well as catch basins and other standing, polluted water sources. Woodland pool habitat following the univoltine species emergence is also an abundant source of *Cx. restuans* in suburban and rural areas. Both species feed mainly on birds and occasionally on mammals, including humans. Peak feeding activity for these species occurs from dusk into the late evening. Consistently high temperatures and lower precipitation rates are factors that have been associated with higher mosquito infection and WNV human illness rates. Additionally, warmer winter temperature conditions may result in larger numbers of *Culex* species overwintering as adults, with resulting increases in early season *Culex* abundance.

*Culex salinarius* has been identified as an important enzootic and epidemic vector of WNV and SLE. *Culex salinarius* thrives in brackish and freshwater wetlands and feeds on amphibians, birds, and mammals. It is well known for biting humans. *Aedes japonicus* may be involved in the transmission of both WNV and EEE. This species uses natural and artificial containers, such as tires and rock pools, as larval habitat. It feeds mainly on mammals and can be an aggressive human biter near the preferred larval habitat.

The role of *Ae. albopictus* in WNV transmission is not well understood, although positive results are reported nearly every year in N.J. *Ae. taeniorhynchus* and *An. quadrimaculatus* have been implicated in SLE transmission.

Mosquitoes become infected with WNV primarily through taking blood meals from infected birds. However, WNV may be passed from infected female mosquitoes to their eggs (i.e., vertical transmission), resulting in infected offspring. Vertical transmission is likely responsible for virus maintenance over the winter in northern parts of the country, but the extent of its contribution to virus amplification and human risk during the peak transmission season is not well understood.

In N.J., WNV has been detected in >25 species with the greatest number of positive species in 2002 (19), and a continued involvement of numerous species when WNV activity was high [2001 (18), 2003 (16), 2012 (10), 2018 (18)].

CMCAs should conduct surveillance for WNV (and SLE) by focusing on:

1. *Cx. pipiens*
2. *Cs. restuans*
3. *Cx. salinarius*
4. *Ae. taeniorhynchus* (for SLE; inefficient for WNV)
5. *An. quadrimaculatus* (for SLE)

Other species that might want to be considered for WNV surveillance include *Ae. albopictus, Ae. japonicus*, and *Cs. melanura*.

<u>WNV & SLE Surveillance - Timing</u>

Surveillance for WNV should be conducted throughout the mosquito surveillance season.

<u>WNV & SLE Surveillance – Geography</u>

WNV is found throughout N.J. and should be a primary focus of surveillance efforts in all counties.

<u>WNV & SLE Trapping</u>

The most used mosquito traps for WNV surveillance sample gravid mosquitoes seeking a place to lay eggs. Gravid traps are highly effective at collecting *Cx. pipiens* and *Cx. restuans*. The advantage of gravid traps is that gravid females have previously taken a blood meal, which greatly increases the likelihood of detecting virus. Gravid traps should be baited with an infusion (“stink water”) which may be comprised of hay, grass clippings, rabbit chow, or other materials that mimic the stagnant water in habitats where these species lay eggs. The different infusions vary in attractiveness. It is, therefore, advisable that infusion preparations are consistent within a surveillance program over time because variations may lead to changes in number and/or type of species captured. Maintaining a consistent effective recipe and infusion mixing routine is recommended. One limitation of gravid traps is that they selectively capture mosquitoes in the *Cx. pipiens* complex, and therefore provide limited information on infection prevalence in other species within a region.

Several other traps such as New Jersey light traps or $CO_2$-baited light traps may be used to collect mosquitoes for WNV monitoring depending on the species or mosquito reproductive state the county wishes to sample. They may also be more effective than gravid traps to detect SLE vectors, *Ae. taeniorhynchus* and *An. quadrimaculatus.* Carbon dioxide-baited CDC miniature light traps may be used to sample potential bridge-vector species including *Aedes, Psorophora*, and *Anopheles* species. The advantage of light traps is that they collect a wide range of mosquito species, which provides information about both primary and secondary vectors and a better understanding of the species composition in an area. A limitation of $CO_2$-baited light traps is that the collections in certain locations and times may consist largely of unfed, nulliparous individuals, which greatly reduces the likelihood of detecting WNV and other arboviruses when only a subset of the trap collections are able to be submitted for testing. Light traps aren't recommended exclusively for WNV & SLE surveillance, however, because not all mosquito species are attracted to light traps and the numbers captured may not reflect the population size of a particular species.

Note: For entry into JerseySurv, CMCAs should enter *Culex* spp. mosquitoes as either the individual *Culex* species when known or as "*Culex pipiens/restuans/salinarius*" (informally referred to as "*Culex* mix") when it is thought that those are the predominant species. "*Culex (*Unspecified)" should only be used if specimens are so poorly preserved that it isn't possible to determine species (NJLT). In general, these wouldn't be submitted for pathogen testing.

WNV & SLE Surveillance Summary:

- Targeted species: *Cx. pipiens, Cx. restuans, Cx. salinarius, Ae. taeniorhynchus, An. quadrimaculatus*
- Timing: Throughout surveillance season
- Geography: All N.J. counties
- Traps: Gravid, $CO_2$-baited light traps, BGS
- Volume: 10-15 pools/week
- Testing: WNV Panel

## Eastern Equine Encephalitis Surveillance

Eastern equine encephalitis virus (EEE), a mosquito-borne alphavirus, is one of the most severe arboviral diseases in North America. Human EEE cases are rare, with an average of 8 cases reported annually in the U.S. between 2003–2018 (range = 4–21 cases/year). However, in 2019, 38 cases were reported nationally. Most cases of EEE have been reported from Atlantic and Gulf Coast states and occur primarily during summer months, with >80% of cases having an illness onset during July through September. Approximately 30% of people with EEE die and many survivors have ongoing neurologic problems. In 2019, 4 human cases of eastern equine encephalitis virus (EEE) were reported in N.J., from Atlantic, Middlesex, Somerset, and Union counties. 2019 was the most active season since the 1959 and 1968 epidemic years where 32 and 12 people respectively contracted the disease. Since 2000, N.J. has reported a total of 8 EEE cases, 3 cases in 2003, 1 case in 2016, and 4 cases in 2019. EEE infection is often fatal in unvaccinated horses as well, making them a useful sentinel for evaluating EEE human risk. The risk of EEE in humans varies by geographical area and is correlated with the location of necessary Atlantic white cedar and red maple swamp larval habitats supporting amplifying and bridge vector species. In N.J. this is typically in the southern part of the state, although human cases and arboviral activity in mosquitoes have more recently been detected in the northern part of the state as well.

### EEE mosquito species of concern

Eastern equine encephalitis virus is maintained in a cycle between *Cs. melanura* mosquitoes and avian hosts in freshwater hardwood swamps. Although infection with EEE can kill some birds, the virus does not kill large proportions of these species. The virus is circulated among the bird populations by *Cs. melanura* and under some circumstances *Cs. morsitans*, another bird-biting mosquito. The virus persists in birds over the winter, and has been suggested to overwinter in reptiles and amphibians, particularly in southern states, but is not known to overwinter in mosquitoes in N.J. Early in the season, a relatively smaller proportion of birds and mosquitoes carry the virus; throughout the mosquito season, continuous transmission between mosquito vectors and bird reservoir hosts increases the proportion of infected birds and mosquitoes leading to an overall greater amount of virus present in the environment.

In the Northeast, the EEE enzootic foci are large swamps of mature Atlantic white cedar and red maple. To grow in the permanently wet swamps, tree roots spread out across the peat soils characteristic of these habitats. These root systems create dark air-filled voids, or crypts, that are flooded with water. These crypts are the preferred oviposition (egg-laying) sites for *Cs. melanura* and are where the larvae develop in the cool, dark, acidic waters. *Culiseta melanura* overwinters as larvae in these environmentally stable protected habitats. The amount of rainfall occurring during the summer and fall of the preceding year affects the survival of the larvae during the winter period and, in part, determines the population of adult mosquitoes successfully emerging the following year.

Depending on when virus circulation begins, the size of the *Culiseta* populations, weather conditions, and probably additional currently unidentified factors, this virus amplification cycle may eventually spill over and involve secondary, or "bridge" mosquito vectors that feed on both birds and mammals. In the Northeast, these bridge vectors include *Ae. vexans, Cq. perturbans, Cx. salinarius* and *Ae. canadensis. Coquillettidia perturbans* is considered to be the most important bridge vector in Massachusetts and is thought to be an inland driver of EEE in N.J. *Aedes sollicitans* plays an important role in coastal cases in N.J., although more data is needed to characterize its role in transmission. *Culex. erraticus* is another possible bridge and endemic vector for transmission in the southeast U.S. *Culex pipiens* and *Cx. restuans* mosquitoes found positive for EEE are not considered to play a significant role in transmission of the virus to humans or animals because research indicates that *Cx. pipiens* clears the virus within 4 days. EEE positive *Cx. pipiens/restuans* may, however, indicate the presence of virus circulating in the area.

*Aedes canadensis* develops in a wide variety of freshwater habitats, including temporary leaf-lined pools in wooded areas, roadside ditches, pools of water in open fields, and permanent swamps. They are univoltine and peak in the beginning of the season and potentially again in August following heavy rainfall events. They primarily feed on mammals, including humans and deer but have also been observed aggressively feeding on reptiles.

In NJ, EEE has been detected in several mosquito species, including: *Ae. albopictus, Ae. canadensis, Ae. japonicus, Ae. taeniorhynchus, Ae, triseriatus, Ae. vexans, An. punctipennis, An, quadrimaculatus, Cq. perturbans, Cs. melanura, Cx. erraticus, Cx. pipiens, Cx. restuans, Cx. Salinarius, and Ur. sapphirina.*

CMCAs should conduct surveillance for EEE by focusing on:

- *Cs. melanura*
- *Cq. perturbans*
- *Cx. erraticus*

Other species that should be considered for EEE surveillance, especially during periods of high EEE activity, include *Ae. sollicitans, Ae. vexans, Ae. canadensis, Ae. triseriatus, An. quadramaculatus,* and *Cx. salinarius.*

EEE Surveillance - Timing

Surveillance for EEE should be conducted throughout the mosquito surveillance season. Note: resting boxes may need to be put out earlier than the start of the season for weathering. New or freshly painted boxes can repel mosquitoes. Surveillance for the three target vectors should be done throughout the mosquito surveillance season, and if positive, other species should be considered for surveillance. Surveillance for EEE in *Cs. melanura* will often go late into the year, given this species' cold hardiness.

EEE Surveillance – Geography

While EEE has been concentrated in the southern part of the state, it has been increasingly detected in northern counties. EEE surveillance should be targeted in all areas of the state. Note: If no EEE has been detected and there are no *Cs. melanura* populations in a county, CMCAs may want to rely on *Culex* species submitted for WNV testing to also capture possible EEE activity.

EEE Trapping

Long-term EEE monitoring using resting box locations have been established in Atlantic (Corbin City), Burlington (Green Bank/Bass River), Camden (Winslow), Cape May (Dennisville) Gloucester (Glassboro), Monmouth (Turkey Swamp), and Salem (Centerton) counties. These sites were established to monitor *Cs. melanura* populations to provide advance warning of EEE activity cycling before the virus spills over into potential bridge vectors like *Aedes sollicitans*. In addition to the State Mosquito Control Commission supported vector surveillance efforts, several county mosquito control agencies have established monitoring locations to collect additional samples from resting boxes and submit them for arboviral testing.

Resting boxes are generally used to sample *Cs. melanura and Cx. erraticus.* Other species (*Ae. vexans, Cq. perturbans, Cx. salinarius, Ae. canadensis*) can be sampled through typical trapping methods such as gravid or un-baited light traps (data from the CDC EEE surveillance program evaluation found un-baited light traps were more effective in attracting *Cs. melanura* compared to those baited with $CO_2$). To maximize *Cs. melanura* collections, county programs are encouraged to use at least one un-baited light trap in addition to resting boxes at sites targeting *Cs. melanura*. These can be placed to sample ponds with emergent vegetation (*Cq. perturbans)*, flooded riverine areas (*Ae. canadensis),* flooded flatlands (*Ae. vexans)* and freshwater impoundments with varying amounts of saltwater (*Cx. salinarius).*

<u>EEE Surveillance Summary:</u>

- Targeted species: *Cs. melanura, Cq. perturbans, Cx. erraticus*
- Timing: Throughout surveillance season
- Geography: All N.J. counties (see note above)
- Traps: Resting boxes with un-baited light trap(s) (preferred), gravid traps
- Volume: 2-5 pools/week (apart from state resting box sites)
- Testing: WNV Panel

**Jamestown Canyon Surveillance**

Human JCV cases are rare, with 166 cases reported between 2004-2018 in the U.S., mostly in the midwestern and northeastern states. Only two human cases have been reported in N.J. in 2015 and 2021, both in Sussex County. White-tailed deer are recognized as a principal amplification host based on experimental infections and presence of antibodies. With increasing reforestation and increases in deer population, this arbovirus may be an increasing concern. It has been isolated from 26 mosquito species, including many Aedes species, and can be vertically transmitted.

Long-term surveillance in Connecticut (1997-2017) detected JCV in 92% of the surveillance sites equally distributed in urban, suburban, and rural locales, with no significant association with land cover categories and no significant relationship with deer abundance.

<u>JCV mosquito species of concern</u>

Long-term surveillance in Connecticut detected JCV in 25 mosquito species, most *Aedes* and *Anopheles*. Five species have been implicated as being of highest importance based on the number of years, sites, and virus detections. These include *Ae. aurifer, Ae. abserratus, Ae. canadensis, Ae. cantator, and An. punctipennis. Aedes canadensis* (Heard, et al, 1991) and *An. punctipennis* have been shown to be competent vectors in laboratory experiments. In Connecticut most virus isolations have been detected most often in *Ae. canadensis* and detected between mid-June and mid-July, consistent with vertical transmission. Long-term maintenance of local populations of JCV suggests overwintering and persistence of virus.

NJ started testing mosquitoes for JCV in 2019. Between 2019-2020, JCV has been identified in 11 pools in seven counties: Bergen, Burlington, Cumberland, Middlesex, Monmouth, Salem, and Sussex, and in seven species: *Ae. abserratus, Ae. cantator, Ae. taeniorhynchus, An. crucians, An. punctipennis, An. quadrimaculatus, Cq. perturbans*. In 2019 pools were detected from MMWR weeks 23-27 (weeks ending June 8- September 14) and in 2020 from MMWR weeks 23-32 (weeks ending June 6 - August 8).

*Aedes canadensis* develop in a variety of freshwater habitats, including temporary leaf-lined pools in wooded areas, roadside ditches, pools of water in open fields and permanent swamps. They are univoltine and peak in the beginning of the season and potentially again in August following heavy rainfall events. They primarily feed on mammals, including humans and deer.

It isn't known which species are responsible for JCV transmission in NJ. Some species of interest may include:

- <u>Species of importance in CT</u>: *Ae. aurifer, Ae. abserratus, Ae. canadensis, Ae. cantator, and An. punctipennis.*
- <u>Positive pools in NJ</u>: *Aedes abserratus, Aedes cantator, Aedes taeniorhynchus, Anopheles crucians, Anopheles punctipennis, Anopheles quadrimaculatus, Coquillettidia perturbans*
- <u>Referenced in literature</u>: *Ae. communis, Ae. punctor, Ae. stimulans, Cs. inornata*

JCV Surveillance - Timing

Surveillance for JCV should be initiated early in the season (late April/early May) and continue through mosquito surveillance season. Because early season species may be collected before the start of the mosquito surveillance season, samples should be frozen and sent to PHEL once routine shipments begin.

JCV Surveillance – Geography

Initial surveillance data has detected JCV sporadically in several regions of the state. Surveillance should be conducted statewide to better characterize the distribution of this emerging arbovirus.

JCV Trapping

Although a variety of trap types have been used in JCV surveillance, $CO_2$-baited light traps have proven most useful for collecting the greatest variety of Aedes species implicated in Jamestown Canyon virus. Traps should be set in areas where the targeted species larval habitat is nearby.

JCV Surveillance Summary:

- Targeted species: Early season *Aedes* species and others as listed above
- Timing: Late April/early May and throughout the season
- Geography: All N.J. counties
- Traps: $CO_2$ baited light traps
- Volume: 2-4 pools/week
- Testing: WNV Panel

**La Crosse Surveillance**

In the United States, an average of 68 La Crosse encephalitis virus (LAC) neuroinvasive disease cases are reported each year, although this number is thought to greatly underestimate the number of total infections, with mild and asymptomatic infections not included. Severe disease occurs most often in children under the age of 16. LAC disease cases occur primarily from late spring through early fall. Historically, most cases of LAC neuroinvasive disease were reported from the upper Midwestern states (Ohio, Wisconsin, Minnesota, Indiana, Illinois, and Iowa). Recently, more cases have been reported from northeastern, mid-Atlantic and southeastern states (North Carolina, Tennessee, West Virginia, Georgia, Virginia, Kentucky, and Rhode Island). N.J. hasn't reported a human LAC case in at least 20 years (but reported 3 cases between 1964 and 2000). LAC is maintained in a cycle between *Ae. triseriatus* (the eastern tree hole mosquito) and vertebrate hosts (especially small mammals such as chipmunks and squirrels) in deciduous forest habitats (i.e., forests with trees that lose their leaves each year).

<u>LAC mosquito species of concern</u>

*Aedes triseriatus* is an aggressive daytime-biting mosquito, especially in or near infested woods. True to its nickname, *Ae. triseriatus* normally lays its eggs in pools of water accumulated in tree holes, but it will also lay eggs in man-made water holding containers, particularly discarded tires. LAC is transmitted vertically, and the virus can survive in dormant eggs through the winter and develop into infected, flying mosquitoes in the spring.

Vertical transmission is a significant mechanism to maintain LAC in nature and horizontal transmission between mosquito vectors and sciurid rodents (i.e., chipmunks, squirrels) allows LAC to persist in the environment and to maintain its ability to amplify successfully in vertebrate hosts. Vector competence studies have identified the capacity for other *Aedes* species to serve as vectors of LAC: *Ae. albopictus, Ae. aegypti* and *Ae. japonicus*. Virus isolation from field-collected *Ae. albopictus* mosquitoes has confirmed their potential to serve as vectors in the field. Although vector competence research revealed poor virus replication in *Ae. canadensis*, field research has shown that they may serve as accessory vectors of LAC. Laboratory studies in Appalachian states have shown that *Culex* species (*Cx. restuans, Cx. pipiens*) may play a role in transmission in endemic areas as well.

In 2019, one mosquito pool (*Ae. triseriatus*) collected in Passaic County tested positive for La Crosse virus at PHEL. This was the first positive pool detected in the state since 2014. In 2014, 2 mosquito pools collected from the Joint Base MDL (Burlington County) by the Department of the Airforce tested positive for LACV (*Ae. triseriatus* and *Ae. albopictus)*.

CMCAs should conduct surveillance for LAC by focusing on:

- *Ae. triseriatus*

Other species that might want to be considered for LAC surveillance include *Ae. albopictus, Ae. canadensis, Ae. hendersoni, Ae. japonicus*.

LAC Surveillance - Timing

Surveillance for LAC should be initiated early in the season (late April/early May) and continue throughout mosquito surveillance season.

LAC Surveillance – Geography

Surveillance data is lacking and should be conducted statewide.

LAC Trapping

The primary vector, *Ae. triseriatus*, is not usually caught in high numbers using conventional trapping methods. Studies going back to the 1960s have used a variety of trapping methods as well as trapping types to maximize catches. It has been reported that *Ae. triseriatus* are not particularly attracted to light traps and may even be reluctant to enter traps baited with light, so the light is often removed from $CO_2$ –baited traps to increase catches. In recent studies, BGS traps baited with $CO_2$ caught higher numbers of *Ae. triseriatus* compared to $CO_2$-baited CDC or gravid traps. Since these traps are set on the ground, this could be partially due to *Ae. triseriatus's* preference to host-seek low to the ground. BGS traps baited with $CO_2$ have the added advantage of also collecting ample numbers of possible secondary vectors of LAC, *Ae. albopictus* and *Ae. japonicus*. Since BGS traps are expensive and cumbersome, many programs do not use them; therefore, CDC traps baited with $CO_2$ with or without light can also be set low to the ground to maximize trap catches of *Ae. triseriatus.* The addition of the BG-lure to either the BGS or CDC trap may increase catch as well, but these lures are relatively expensive and their attractiveness to *Ae. triseriatus* is not well proven. Recent research has shown R-1-octen-3-ol with ammonium bicarbonate to work as an effective lure for *Ae. triseriatus*, but these lures are not commercially available.

Adults are often found in wooded, shaded areas where suitable egg-laying habitat can be found. Since human cases of La Crosse peak in August and September, mosquito surveillance for this disease should begin in late May or June, when *Ae. triseriatus* populations are building.

LAC Surveillance Summary:

- Targeted species: *Ae. triseriatus*
- Timing: Late April/early May through end of surveillance season
- Geography: All N.J. Counties
- Traps: $CO_2$-baited BGS, $CO_2$- baited light traps (set low to the ground)
- Volume: 1-2 pools/week
- Testing: LAC Panel

## Exotic Aedes Species Surveillance

Several travel-associated arboviral human cases are reported in N.J., including chikungunya, dengue, and Zika. Most human cases in N.J. occur in Bergen, Hudson, Passaic, Middlesex, Essex, and Mercer counties, which correlates with concentrations of population groups that travel to countries with endemic activity. These arboviruses are transmitted primarily by exotic *Aedes* species, namely *Ae. aegypti*, which is not established in N.J, and *Ae. albopictus*, which is also a competent vector and found throughout the state. In response to the Zika outbreak in 2016-17, mosquito surveillance shifted to include *Ae. albopictus* in addition to species implicated in WNV and EEE transmission. While there are locally acquired infections reported in Florida and Texas, the primary surveillance goals in N.J. are to detect new introductions of *Ae. aegypti*, to understand the distribution of *Ae. albopictus*, and to detect travel associated *Aedes* infections that might be imported into N.J. via human infection post-travel.

### *Aedes* mosquito species of concern

A couple of *Ae. aegypti* mosquitoes have been detected in NJ, but established populations have not been identified. CMCAs that do identify *Ae. aegypti* should notify SMCC.

*Aedes albopictus* (the Asian tiger mosquito) is a container-inhabiting species which lays its eggs in any water-containing receptacle in urban, suburban, rural, and forested areas. The primary immature habitats of this species are artificial containers such as tires, flowerpots, cemetery urns/vases, buckets, tin cans, rain gutters, ornamental ponds, and drums. Larvae are also found in natural containers such as tree holes, bamboo pots and leaf axils. It is a very aggressive daytime biter with peak activity generally occurring during the early morning and late afternoon, feeding on a variety of hosts including humans, domestic and wild animals, and birds. Its generalized feeding behavior contributes to its vector potential. In the lab, northeastern strains of *Ae. albopictus* were shown to be potential vectors of Zika virus and dengue virus 2 and highly competent vectors of chikungunya virus. *Aedes albopictus* has been detected in all NJ counties, but abundance levels in each county is not known.

### Surveillance

All counties should conduct targeted surveillance for *Ae. albopictus* to monitor abundance trends over time using a minimum of 1-2 BGS traps. Abundance data should be reported in JerseySurv.

In addition, limited adult trapping programs for exotic species such as *Ae. aegypti* is advisable in areas with the highest likelihood of introduction such as airports, seaports, and any areas known to receive bulk goods from overseas. All ID staff should be trained to identify exotic species in case they occur in any of the routine surveillance efforts.

In the event *Ae. aegypti* is detected, trapping should be immediately increased radially from the point of detection to determine if it is a single introduction or to measure the geographic extent of an established population. Extensive larval surveillance and control should also occur in the area to determine the potential source of the introduction and limit the spread of the species. The CMCA should also alert the SMCC of the detection so that assistance can be provided and coordinated across counties if needed.

Pathogen Testing

It should be noted that even in areas of the US with endemic human transmission, detection of these diseases in the mosquito population is extremely rare. Therefore, testing resources should be prioritized for:

- Response to imported human cases, particularly clusters of imported cases, OR
- Response to human cases with no international travel history, AND
- When *Ae. albopictus* is detected near the residence of human cases.

In the event of locally acquired human cases of disease, the SMCC & NJDOH would provide additional surveillance and/or control guidance.

Exotic Aedes Species Surveillance - Timing

Surveillance for exotic *Aedes* species should be conducted throughout the mosquito surveillance season and in response to human case notification when indicated.

Exotic Aedes Species Surveillance - Geography

Routine surveillance for exotic *Aedes* species should be conducted statewide, with additional surveillance conducted based on emerging needs.

Exotic Aedes Species Surveillance Trapping

BGS, CDC trap

Exotic Aedes Surveillance Summary:

- Targeted species: *Ae. albopictus, Ae. aegypti*
- Timing: Throughout surveillance season, and/or in response to human cases
- Geography: Statewide surveillance; focused testing only
- Traps: BGS
- Surveillance: 1-2 traps/week
- Testing Volume: No minimum; in response to human case notifications, or local needs
- Testing: Exotic Aedes Panel

## Malaria Surveillance

There are >2,000 cases of imported malaria reported in the U.S. each year. Three-quarters of cases occur among persons visiting family and friends in malaria endemic areas and 95% of cases didn't complete a full course of malaria prophylaxis. Between 2010-2022, there were an average of 86 imported malaria cases reported in N.J. each year. N.J. is one of the states with the highest number of imported malaria cases in the U.S. Cases have been reported in all counties except Salem and Cape May and the highest number of cases are reported in Essex, Middlesex, Hudson, and Mercer counties. Most imported cases are associated with travel to Africa, with a lesser number associated with travel to Asia and Latin America. Most malaria cases in NJ are infected by *Plasmodium falciparum*, followed by *P. vivax.*, although all malaria species have been reported.

The risk of locally-acquired malaria is very low in the U.S. Between 1957 and 2003, CDC documented 63 incidents of locally acquired malaria that resulted in 156 total cases. In 2023, locally-acquired cases were reported in Florida, Texas, Maryland, and Arkansas. These were the first locally-acquired cases in the U.S. since 2003. Locally-acquired malaria cases were last reported in N.J. in 1991, with 2 cases identified in Camden and Monmouth counties. Prior to 1991, locally-acquired cases hadn't been identified in N.J. since the 1960s. Given the high number of imported cases, a significant number of international travelers, and competent mosquito vectors, it is important to maintain vigilance for potential locally acquired cases.

Surveillance for malaria relies on prompt public health investigation of human malaria cases, including ascertainment of a relevant travel history to a malaria endemic area. Should a locally-acquired case be suspected, public health officials would work with healthcare and vector control partners to conduct enhanced human and mosquito surveillance to prevent further transmission.

### *Anopheles* mosquito species of concern

Several *Anopheles* mosquitoes that can transmit malaria are present in N.J., with *Anopheles quadrimaculatus* thought to be the primary vector of concern in the eastern U.S.  Other potential vectors include Anopheles crucians (complex), which includes *An. bradleyi, An. punctipennis*, and *An. quadrimaculatus*. Anophelines often live in marshy areas or along the banks of creeks or streams. Most anophelines bite in the evening or middle of the night, so if control is warranted, spray activities should coincide with the peak activity-time of the local species.

### Surveillance

***Routine***: CMCAs should survey and map annually all actual and potential Anopheline larval habitats in the county; monitor Anopheline adult distribution and seasonal abundance collections in JerseySurv; and be aware of areas in the county with a high number of imported malaria cases.

***In response to a suspected locally-acquired case***: If it is determined that a malaria case could be locally acquired, mosquito trapping should be performed in the immediate vicinity of the residence of the case-patient and within a radius of at least 0.6 miles (1 km). Enhanced mosquito surveillance/trapping should be conducted for at least 6 weeks from the time of diagnosis. This timeframe should be sufficient to capture any mosquitoes that could have potentially bitten the case (if there are >1 case, then the 6-week timeline should extend from the date of diagnosis of the last case. If areas are identified where the case spent significant time outdoors, enhanced mosquito surveillance should be performed around those areas as well as the residence.

Pathogen Testing

***Routine***: Routinely testing of *Anopheles* mosquitoes for *Plasmodium* species is not currently recommended.

***In response to a suspected locally-acquired case:*** All collected anophelines should be sent to PHEL for forwarding to CDC for *Plasmodium* species testing. Current CDC protocols include testing the head/thorax to detect *Plasmodium* sporozoites (to identify infectious mosquitoes) and testing the abdomen to detect *Plasmodium* DNA (to identify mosquitoes that may have fed on an infected host).

In the event of a locally acquired human case, the SMCC & NJDOH would provide additional surveillance and/or control guidance.

*Anopheles* Species Surveillance - Timing

Surveillance for anophelines should be conducted throughout the mosquito surveillance season and in response to a suspected local case.

*Anopheles* Species Surveillance - Geography

Routine surveillance for anophelines should be conducted statewide, with additional surveillance conducted based on a suspected locally acquired case.

*Anopheles* Species Surveillance Trapping

CDC Light traps, gravid traps, and resting boxes

Malaria Surveillance Summary:

- Targeted species: *Anopheles quadrimaculatus, An. punctipennis*, *An, crucians*, *An. bradleyi*
- Timing: Routine surveillance throughout mosquito season; enhanced surveillance In response to suspected locally-acquired human case
- Geography: Routine surveillance throughout mosquito season; enhanced surveillance In response to suspected locally-acquired human case
- Traps: Resting box, CDC, gravid
- Surveillance: As indicated, in response to suspected locally-acquired human cases
- Testing Volume: No minimum; in response to suspected locally-acquired human cases
- Testing: To be determined at CDC

## Appendix A: Mosquito Species of Interest and Habitat

***Aedes abserratus*** is an early season univoltine species that is most common in northwestern New Jersey, but that can be found in any area with suitable larval habitat, including freshwater bogs and marshes. It is primarily a mammal-biting mosquito that feeds on humans and can be a nuisance after dark. However, because of its early season activity and spotty distribution, it is generally a minor pest species in New Jersey. This species may be involved in the transmission of Jamestown Canyon virus.

***Aedes aegypti*** is a container-inhabiting species that is well-adapted to urban or domesticated habitats in the southern USA. It is the vector of Zika, dengue, chikungunya, and yellow fever viruses. Although individual mosquitoes have occasionally been found, there are no reports of established populations in New Jersey. *Ae. aegypti* has also been implicated in LAC transmission.

***Aedes albopictus*** (the Asian tiger mosquito) is a container-inhabiting species which lays its eggs in any water-containing receptacle in urban, suburban, rural, and forested areas. The primary immature habitats of this species are artificial containers such as tires, flowerpots, cemetery urns/vases, buckets, tin cans, rain gutters, ornamental ponds, and drums. Larvae are also found in natural containers such as tree holes, bamboo pots and leaf axils. It is a very aggressive daytime biter with peak activity generally occurring during the early morning and late afternoon, feeding on a variety of hosts including humans, domestic and wild animals, and birds. The role of *Ae. albopictus* in WNV transmission is not well understood, although positive results are reported nearly every year in N.J. *Ae. albopictus* is a vector of Chikungunya, Dengue, and Zika and may be associated with LAC transmission as well.

***Aedes aurifer*** is an early season species with a single generation of larvae that are present between April and May. The species is more common in the northern half of the state but are locally abundant as far south as Atlantic County. Larval habitat includes freshwater swamps, bogs, or woodland pools that intergrade with a freshwater swamp source and have emergent vegetation. The species will readily feed on humans, but it is a minor pest in New Jersey because of its limited numbers and early season activity. *Ae. aurifer* may be associated with JCV transmission.

***Aedes canadensis*** develops in a wide variety of freshwater habitats, including temporary leaf-lined pools in wooded areas, roadside ditches, pools of water in open fields, and permanent swamps. They are univoltine and peak in the beginning of the season and potentially again in August following heavy rainfall events. They primarily feed on mammals, including humans and deer but have also been observed aggressively feeding on reptiles. *Ae. canadensis* is thought to be a bridge vector for EEE and may be involved in transmission of JCV and LAC.

***Aedes cantator*** is a multivoltine salt marsh species that occurs in greatest numbers during the spring. Larvae are present along the upland edge of salt marsh habitats and generally peak in mid-May. As the season advances, larvae appear in lesser numbers and become mixed with *Aedes sollicitans*, although they can generally be collected well into the fall. Inland populations are often found in floodwater areas that receive runoff from highways that are salted during the winter for ice removal. *Aedes cantator* is a significant pest because high population numbers may be present in coastal areas. *Ae. cantator* may also be involved in JCV transmission.

***Aedes communis*** is a true snow pool species and is one of the earliest mosquitoes to appear in New Jersey. Larvae are present in March and begin pupating during the 3rd week of April. The mosquito is found in mountainous habitat in New Jersey where few homes exist and is not generally a significant pest. Jamestown Canyon virus has been isolated from larvae, suggesting *Ae. communis* may play a role in the maintenance cycle of this virus.

***Aedes hendersoni*** is a multivoltine freshwater species closely related to *Aedes triseriatus* in both morphology and larval habitat. Their ranges overlap in the northeastern USA. *Aedes hendersoni* is a natural container inhabiting mosquito which can be found using deep rotted holes in maple trees higher up in the canopy. Because this species can be difficult to separate from *Aedes triseriatus,* a very common mosquito in New Jersey, reports of this species tend to be rare. *Aedes hendersoni* has been reported to feed predominantly on small mammals including tree squirrels and racoons. Its role in transmission of LAC remains unclear.

***Aedes japonicus*** uses natural and artificial containers, such as tires and rock pools, as larval habitat. It feeds mainly on mammals and can be an aggressive human biter near the preferred larval habitat. *Aedes japonicus* may be involved in the transmission of WNV, EEE, and LAC.

***Aedes punctor*** is a univoltine northern *Aedes* species with a typical northern life cycle type. Eggs are deposited singly in leaf lined depressions with moist earth at higher elevations that will become flooded with water in the spring. Larvae occur most often in heavily wooded areas early in the spring in temporary pools of cool acidic water. Adults, which are long lived, are most abundant in May and can occasionally be collected through July and into August in cold heavily shaded dense forest habitats. *Aedes punctor* primarily feeds on large mammals and can be a significant pest feeding during daylight hours and after dark. *Ae. punctor* may also be involved in JCV transmission.

***Aedes sollicitans*** is a major pest species in many areas on the east coast. Besides being a serious nuisance, the eastern saltmarsh mosquito is an important vector of Eastern equine encephalitis, Venezuelan equine encephalitis, and dog heartworm. *Aedes sollicitans* larvae develop in pools and puddles that are produced by high tides and/or heavy rainfalls. Since these aquatic systems tend to dry up quickly, emergence of adult *Ae. sollicitans* can occur in as little 4 to 5 days following egg hatch. Females obtain blood primarily from mammals and to a lesser extent from birds. In New Jersey *Ae. sollicitans* adults are most abundant in the summer into early fall. *Ae. sollicitans* plays an important role in coastal cases in N.J., although more data is needed to characterize its role in EEE transmission.

***Aedes stimulans*** is an early season univoltine species that is widely distributed in New Jersey but is more common in the northern third of the State. Larvae are primarily found in shaded woodland pools with a lining of leaf litter. *Aedes stimulans* is an important early season pest because females may enter residential areas in high numbers in search of a blood meal. *Aedes stimulans* is exceptionally long lived and can be collected in light traps into the month of August. Because of its longevity, the species is considered a probable vector of dog heartworm in northern New Jersey and may be involved in JCV transmission.

***Aedes taeniorhynchus*** is an aggressive human biter that is most common along the southern coasts from North Carolina to the Caribbean. It has been shown to transmit eastern equine encephalitis and St. Louis encephalitis in the laboratory. It is present throughout coastal New Jersey, but usually less common than *Ae. sollicitans.* Like *Ae. sollicitans*, larval habitat includes grassy salt marshes associated with spike grass (*Distichlis spicata*) and salt meadow hay (*Spartina patens*). This mosquito will feed on birds as well as mammals.

***Aedes triseriatus*** is an aggressive daytime-biting mosquito, especially in or near infested woods. True to its nickname, *Ae. triseriatus* normally lays its eggs in pools of water accumulated in tree holes, but it will also lay eggs in man-made water holding containers, particularly discarded tires. It is the primary vector of La Crosse virus, which is transmitted vertically; La Crosse virus can survive in dormant eggs through the winter and develop into infected, flying mosquitoes in the spring. *Ae. triseriatus* may play a role in EEE transmission.

***Aedes vexans*** is a multivoltine mosquito that is found in a wide variety of freshwater habitats, often following rains. Females will feed during the day but are most active at dusk. In New Jersey, emergence of *Ae. vexans* usually begins in mid-May and populations often reach nuisance levels early in June. Adults may be present into October when autumn temperatures remain warm. Transmission of St. Louis encephalitis has been demonstrated in the laboratory. *Ae. vexans* is thought to be a bridge vector for EEE.

***Anopheles crucians*** is part of a complex of *Anopheles* species including *An. bradleyi, An. georgianus,* and several others that are nearly indistinguishable as adults. *Anopheles crucians* is the freshwater species found in a variety of aquatic habitats including ponds, swamps and marshes containing acidic water. The mosquito overwinters as a larva in New Jersey. Because these adult mosquitoes are difficult to separate from the salt tolerant species *An. bradleyi,* which is also found in New Jersey, adult samples are often speciated by their proximity to known larval habitat. Adults are reported to feed on a wide variety of large and small mammals including humans. They have also been reported to feed on chickens. Members of the *An. crucians* complex were important vectors of malaria. Anopheles crucians is considered a potential vector for EEE, SLE, and WNV based on isolations from this species.

***Anopheles punctipennis*** is a common multivoltine freshwater species that can survive in a wide variety of semi-permanent to permanent water larval habitats. These would include both natural and artificial containers, ponds, pools in intermittent streams, rainwater filled puddles, deep tire ruts, flooded pastures and roadside ditches sometimes containing a significant amount of organic matter. This mosquito feeds primarily on mammals, both small and large, but will occasionally feed on birds. It has been reported to be an important vector of human malaria and may also be associated with JCV and EEE virus transmission.

***Anopheles quadrimaculatus*** was an important vector of malaria in the southeastern United States and today is a major host of the nematode that causes dog heartworm. The species is widespread in New Jersey and is a significant pest of humans and livestock. Larvae are found in a wide variety of clean water habitats, including reservoirs, lakes, rivers, and swamps. *An. quadrimaculatus* is an important vector of human malaria and have been implicated in SLE and may be involved in EEE transmission.

***Coquillettidia perturbans*** is a significant nuisance mosquito in New Jersey and is thought to be involved in transmission of eastern equine encephalitis virus in New Jersey. Larvae use a modified air tube to attach themselves to the roots and stems of aquatic plants where they remain throughout development. *Cq. perturbans* is thought to be a bridge vector for EEE.

***Culex pipiens*** may be abundant in urban areas inhabiting artificial containers (e.g., birdbaths, discarded tires, buckets, clogged gutters) as well as catch basins and other standing, polluted water sources. It feeds mainly on birds and occasionally on mammals, including humans. Peak feeding activity occurs from dusk into the late evening. Warmer winter temperature conditions may result in larger numbers of *Culex* species overwintering as adults, with resulting increases in early season *Culex* abundance. *Cx pipiens* is a primary vector of WNV and SLE.

***Culex restuans*** may be abundant in urban areas inhabiting artificial containers (e.g., birdbaths, discarded tires, buckets, clogged gutters) as well as catch basins and other standing, polluted water sources. Woodland pool habitat following the univoltine species emergence is also an abundant source of *Cx. restuans* in suburban and rural areas. They feed mainly on birds and occasionally on mammals, including humans. Peak feeding activity occurs from dusk into the late evening. Warmer winter temperature conditions may result in larger numbers of *Culex* species overwintering as adults, with resulting increases in early season *Culex* abundance. *Cx restuans* is a primary vector of WNV and SLE.

***Culex salinarius*** thrives in brackish and freshwater wetlands and feeds on amphibians, birds, and mammals. It is well known for biting humans. *Culex salinarius* has been identified as an important enzootic and epidemic vector of WNV and SLE and may be implicated in transmission of EEE.

***Culiseta inornata*** is a multivoltine freshwater species in the *Culex pipiens* life cycle type. Larvae can be collected from a wide variety of freshwater habitats including foul, deoxygenated water, woodland pools, snow melt pools and roadside ditches with emergent vegetation. Dredge spoil habitats also support populations of this species in New Jersey. Females will feed readily on large and small mammals and have been reported to feed on domestic chickens. Several arboviruses, including WNV, have been isolated from this species and it should be considered a potential vector.

***Culiseta melanura*** live in large swamps of mature Atlantic white cedar and red maple. To grow in the permanently wet swamps, tree roots spread out across the peat soils characteristic of these habitats. These root systems create dark air-filled voids, or crypts, that are flooded with water. These crypts are the preferred oviposition (egg-laying) sites for *Cs. melanura* and are where the larvae develop in the cool, dark, acidic waters. *Cs. melanura* overwinters as larvae in these environmentally stable protected habitats. The amount of rainfall occurring during the summer and fall of the preceding year affects the survival of the larvae during the winter period and, in part, determines the population of adult mosquitoes successfully emerging the following year. Eastern equine encephalitis (EEE) virus is maintained in a cycle between *Cs. melanura* mosquitoes and avian hosts in freshwater hardwood swamps.

## Appendix B: Trap Considerations

There are many different trap types available for mosquito surveillance. Each trap type comes with its own built-in bias which is largely based on the choice of attractant used. Examples would include traps that use light, color, $CO_2$, a gravid trap infusion, and or a specific lure. The micro habitat where the trap is placed is also an extremely important factor to consider with respect to the mosquitoes that may be collected (e.g., a trap placed in a shaded forested habitat). Attractants aside, placing the trap on the ground, at chest height or up in the canopy will often produce significantly different results. Other variables within trap types include different “brands” of traps that are available on the market and that use a specific attractant.

Significant differences in trap collections can occur between these various designs. The differing components used to build the trap (batteries, motors, fans, tubes, collection vessels) introduce a seemingly endless source of variation. Combining attractants is yet another significant source of variability with respect to accurately reporting repeatable routine surveillance efforts. Examples here might include a BGS trap (contrasting color is one design source of attractant) with the addition of $CO_2$. This is now a $CO_2$ baited trap without a light source sitting on the ground. Should a lure also be added the variables continue to increase and should be documented. JerseySurv allows the user to document and track attractants used with the various traps available (see table below).

Within the mosquito control community, there have been ongoing efforts to build a better trap (more efficient, less expensive, better fit for the program) to meet local needs. Perhaps the most important consideration for a mosquito surveillance program is to decide on a trap type for a specific purpose; ensure that traps are performing consistently through regular calibration checks and routine maintenance; and sustain a consistent approach over time. Should changes be incorporated into a trapping program, documenting these changes at the time they are made is very important and extremely helpful when analyzing historical data trends.

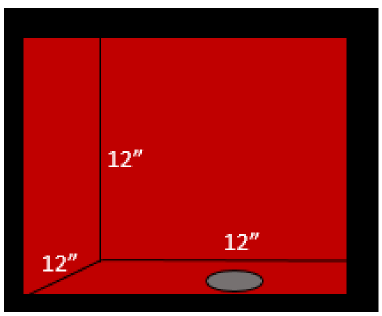
| Trap Name | JerseySurv Acronym | Available Attractants |
|---|---|---|
| American Biophysics Corporation (ABC) trap | CO2-ABC | Light, CO2, Lure |
| Backpack aspirator | BACKPACK | None |
| BG Sentinel | BGSENT | CO2, BG-Lure, BG-Sweetscent, Octenol |
| Biogents Counter Trap | BGCOUNTER | CO2, Lure |
| Biogents Gravid Aedes Trap | BGGAT | Infusion |
| Biogents Pro - CDC-style | BGPRO-CDC | Light, CO2, Lure |
| Biogents Pro - EVS-style | BGPRO-EVS | Light, CO2, Lure |
| Biogents Pro - Sentinel-style | BGPRO-SENT | Light, CO2, Lure |
| Bird baited trap | BBT | Avian host |
| Carbon dioxide baited trap | CO2 | CO2 |
| CDC Autocidal Gravid Ovitrap (counts) | CDCAGO | Infusion |
| CDC Autocidal Gravid Ovitrap (presence/absence) | CDCAGO-PRES | Infusion |
| CDC Fay-Prince Trap | FAYPRINCE | Motion, Light, CO2 |
| CDC miniature light trap | CO2-CDC | Light, CO2, Lure |
| CDC-style Gravid Trap | GRVD-CDC | Infusion |
| Collection Bottle Rotator Trap | CO2-ROTAT | Light, CO2, Lure |
| EVS (Encephalitis Vector Survey) trap | CO2-EVS | Light, CO2, Lure |
| Frommer Updraft Gravid Trap | GRVD-FROMM | Infusion |
| Gravid trap | GRVD | Infusion |
| Hand or another manual aspirator | ASPIRATOR | Human, None |
| Human Landing Counts | LANDING | Human, None |
| Mammal baited trap | MBT | Mammalian host |
| Modified CDC Autocidal Gravid Ovitrap (counts) | MODAGO | Colors, Infusion |
| Mosquito magnet trap | MMT | CO2, Lure |
| Nasci Backpack Aspirator | BACKPACK-N | None |
| New Jersey light trap | NJLT | Light, CO2, Lure |
| Omni-Directional Fay-Prince Trap | OMNIFAY | Light, CO2, Lure |
| Prokopack Aspirator | PROKOPACK | None |
| Reiter-Cummings Gravid Trap | GRVD-RC | Infusion |
| Resting box collection | REST | Colors |
| Sweep Net | SWEEPNET | Human, None |
| UV Light Trap | UVLT | Light, CO2, Lure |

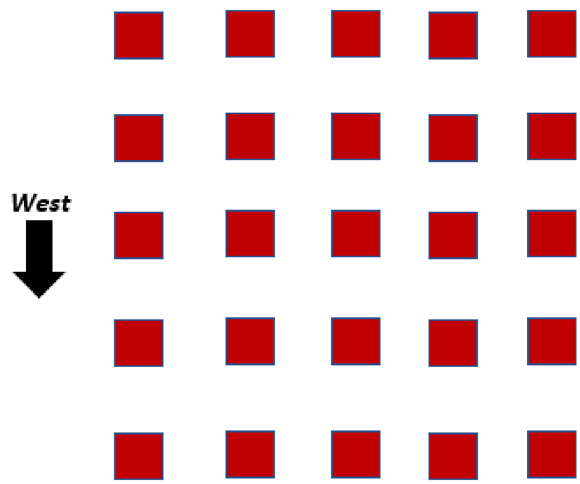
## Appendix C: New Jersey Resting Boxes & Collection Methods Protocol

For full instructions, please refer to the original document from which this guide was abridged:
Crans, Wayne J. (1995). Resting Boxes as Mosquito Surveillance Tools. *Proceedings of the 82nd Annual Meeting of the NJ Mosquito Control Association, Inc.* pp. 53-57. https://vectorbio.rutgers.edu/outreach/restbox.htm

*All State resting box sites MUST be collected using this methodology. It is strongly encouraged that County-run sites also be collected in this manner to provide state-wide consistency in data comparison.*

**Description:** Resting Boxes are 1 cubic foot wooden boxes which are painted black on the outside and red on the inside. The boxes should be placed in groups of 25 or 50 in thick forested areas with high canopy, adjacent to (not within) *Culiseta melanura* habitat. Place the boxes with the open-end facing West. The screened hole should be on the ground to increase humidity. Boxes should be 10 feet from one another, in a line or grid design.




If space does not allow for a grid, lines are acceptable.

**Collections:** Collections should be made between the hours of 10am and 2pm. Items to have on hand:

- Catch cover – this is to cover the end of the box & may be made of cloth, wood, or plexiglass.
- Spray – anesthetic (T.E.A., starter fluid, chloroform, etc.).
- Aspirator – mechanical or mouth (forceps may also be used).

**Method**:

1. Cover the opening of the box with the catch cover (wood, plexiglass, cloth) and tip the box to expose the screened hole.
2. Spray a few short bursts of the anesthetic into the screened hole.
3. Move to the next box and repeat (up to 3 boxes for knockdown).
4. Go back to the 1st box, remove the catch cover, and collect all the mosquitoes using the aspirator/ forceps.
5. Move to the 3rd box and repeat steps 1 & 2.
6. Go back to box 2 and collect the mosquitoes.
7. Repeat until all boxes have been collected.

**JerseySurv data entry:**

- Number of nights should always be 1 (the mosquitoes are not trapped but are free to come and go; even if you haven't collected in 7 days, the number of nights will still be 1).
- Number of traps: this number depends on the program. Most state-run RB sites are in groups of 25 or 50. This number should reflect the number of boxes that you collected from during that trap collection. For example, if you collected from 25 boxes this morning, you'd enter 25 in the "number of traps" in the abundance portal.

## Acknowledgements

These guidelines were developed and updated by a subgroup of the New Jersey Vector-borne Disease Working Group (VBDWG). Sincere thanks to all who contributed their time and expertise to develop and produce this guidance document.

- Kim Cervantes, New Jersey Department of Health, Chair, VBDWG
- Autumn Angelus, New Jersey Department of Environmental Protection, Office of Mosquito Control Coordination
- Matt Bickerton, Bergen County Department of Health Services
- Scott Crans, New Jersey Department of Environmental Protection, Office of Mosquito Control Coordination
- Jennifer Gruener, Warren County Mosquito Extermination Commission
- Greg Williams, Hudson Regional Health Commission
- Nicholas Indelicato, Mercer County Mosquito Control
- Tadhgh Rainey, Hunterdon County Division of Mosquito & Vector Control
- Lisa Reed, Center for Vector Biology, Rutgers University

## References:

Andreadis, T. G., Anderson, J. F., Armstrong, P. M., & Main, A. J., (2008), Isolations of Jamestown Canyon virus (Bunyaviridae: *Orthobunyavirus*) from field-collected mosquitoes (Diptera: Culicidae) in Connecticut, USA: a ten-year analysis, 1997-2006.*Vector Borne Zoonotic Diseases, 8*(2)*,* 175-88 https://doi.org/10.1089/vbz.2007.0169

Andreadis, T.G. & Armstrong, P. M. (2007). A two-year evaluation of elevated canopy trapping for Culex mosquitoes and West Nile virus in an operational surveillance program in the northeastern United States. *Journal of the American Mosquito Control Association*, *23*, 137-148. https://doi.org/10.2987/8756-971X(2007)23[137:ATEOEC]2.0.CO;2

Arrigo, N. C., Adams, A. P., Watts,D. M., Newman, P. C., & Weaver, S. C. (2010). Cotton rats and house sparrows as hosts for North and South American strains of eastern equine encephalitis virus. *Emerging Infectious Diseases*, *16*(9), 1373. https://wwwnc.cdc.gov/eid/article/16/9/10-0459_article

Barr, A. R., Morrison, A. C., Guptavanij, P., Bangs, M. J., & Cope, S. E. (1986). Parity rates of mosquitoes collected in the San Joaquin Marsh. *Proceedings and papers of the annual conference of the California Mosquito and Vector Control Association (USA)*. http://agris.fao.org/agris-search/search.do?recordID=US8745550 [Accessed December 7, 2017]. https://www.mvcac.org/amg//wp-content/uploads/1986-MVCAC-Volume-54.pdf (page 117)

Berry, R.L., Parsons, M. A., LaLonde-Weigert,, B. J.,Lebio, J., Stegmiller, H., & Bear, G. T. (1986). *Aedes canadensis*, a vector of La Crosse virus in Ohio. *Journal of the American Mosquito Control Association*, *2*(1), 73-78. Link

Bidlingmayer, W. L. (1967). A comparison of trapping methods for adult mosquitoes: species response and environmental influence. *Journal of Medical Entomology*, *4*, 200-220.https://doi.org/10.1093/jmedent/4.2.200

Bingham, A. M., Graham, S. P., Burkett-Cadena, N. D., White, G. S., Hassan, H. K., & Unnasch, T. R. (2012). Detection of eastern equine encephalomyelitis virus RNA in North American snakes. *The American Journal of Tropical Medicine and Hygiene*, *87(6)*, 1140-1144. Link

Burkett, D. A., Kelly, R., Porter, C. H., & Wirtz, R. A. (2004). Commercial mosquito trap and gravid trap oviposition media evaluation, Atlanta, Georgia. *Journal of the American Mosquito Control Association, 20*(3), 223- 228. https://europepmc.org/article/med/15532919

Centers for Disease Control and Prevention: Eastern Equine Encephalitis Virus Surveillance and Control Guidelines. Retrieved from https://www.cdc.gov/mosquitoes/guidelines/eastern-equine-encephalitis/introduction.html. Link

Centers for Disease Control and Prevention: West Nile Virus Surveillance and Control Guidelines. Retrieved from https://www.cdc.gov/mosquitoes/guidelines/west-nile/introduction.html. Link

Connecticut Agricultural Experiment Station: Jamestown Canyon Virus Revisited, Are We Neglecting an Under-recognized Mosquito-borne disease. Retrieved from https://portal.ct.gov/-/media/CAES/Mosquito-Testing/Mosquito-Borne-Disease-Symposium-2019/Andreadis_CAES_Jaestown-Canyon-Virus.pdf Link

Clark, G. G., Crans, W. J., & Crabbs, C. L. (1985). Absence of eastern equine encephalitis (EEE) virus in immature *Coquillettidia perturbans* associated with equine cases of EEE. *Journal of the American Mosquito Control Association*, *1*(4), 540-542. Link

Crans, W. J. (1977). The status of *Aedes sollicitans* as an epidemic vector of eastern equine encephalitis in New Jersey [Insects]. *Mosquito News*. Link

Crans, W. J. (1982). The use of sentinel chickens for St Louis encephalitis surveillance in New Jersey. *Proceedings of the Annual Meetings of the New Jersey Mosquito Control Association, 69*, 39-41. Link

Crans, W. J. (1986). Failure of chickens to act as sentinels during an epizootic of eastern equine encephalitis in southern New Jersey, USA. *Journal of medical entomology*, *23*(6), 626-629. Link

Crans, W. J., & McCuiston, L. J. (1993). New Jersey's approach to encephalitis prevention. *Bulletin of the Society for Vector Ecology*, *11,* 178-184. Link

Crans, W. J., & Rockel, E. G. (1968). The mosquitoes attracted to turtles. *Mosquito News*, *28*(3), 332-337. Link

Crans, W. J., & Schulze, T. L. (1986). Evidence incriminating *Coquilletidia perturbans* (Diptera: Culicidae) as an epizootic vector of eastern equine encephalitis. I: Isolation of EEE virus from *Cq. perturbans* during an epizootic among horses in New Jersey. *Bulletin of the Society for Vector Ecology*, *11*(1), 178-184. Link

Crans, W. J., & Sprenger, D. A. (1996). The blood-feeding of *Aedes sollicitans* (Walker) in relation to eastern equine encephalitis virus in coastal areas of New Jersey. III. Habitat preference, vertical distribution, and diel periodicity of host-seeking adults. *Journal of Vector Ecology*, *21*(1), 6-13. Link

Crans, W. J., Lesser, F., & Candeletti, T. (1979). Recent distribution records of *Culex tarsalis* in New Jersey. *Mosquito News*, *39*(2), 244-247. Link

Crans, W. J., McCuiston, L. J., & Sprenger, D. A. (1990). The blood-feeding habits of *Aedes sollicitans* (Walker) in relation to eastern equine encephalitis virus in coastal areas of New Jersey. I. Host selection in nature determined by precipitin tests on wild-caught specimens. *Bulletin of the Society for Vector Ecology*, *15*(2), 144-148. Link

Crans, W. J., McNelly, J., Schulze, T. L., & Main, A. (1986). Isolation of eastern equine encephalitis virus from *Aedes sollicitans* during an epizootic in southern New Jersey. *Journal of the American Mosquito Control Association*, 2(1), 68-72. Link

Crans, W. J., Sprenger, D. A., & Mahmood, F. (1996). The blood-feeding habits of *Aedes sollicitans* (Walker) in relation to eastern equine encephalitis virus in coastal areas of New Jersey. II. Results of experiments with caged mosquitoes and the effects of temperature and physiological age on host selection. *Journal of vector ecology*, *21*(1), 1-5. Link

Downing, J. D., & Crans, W. J. (1977). The Ehrenberg pigeon trap as a sampler of Culex mosquitoes for St. Louis encephalitis surveillance. *Mosquito News*. Link

Dye-Braumuller KC, Kanyangarara M. Malaria in the USA: How Vulnerable Are We to Future Outbreaks? Curr Trop Med Rep. 2021;8(1):43-51. doi: 10.1007/s40475-020-00224-z. Link

Eastwood, G., Donnellycolt, A. K., Shepard, J. J, Misencik, M.,J, Bedoukian, R., Cole, L., Armstrong, P. M., & Andreadis, T. G. (2020). Evaluation of novel trapping lures for monitoring exotic and native container-inhabiting *Aedes* spp. (Diptera: Culicidae) mosquitoes. *Journal of Medical Entomology*, *57*(2),534-541. doi: 10.1093/jme/tjz200. PMID: 31875224 Link

Ebsary, B. A., & Crans, W. J. (1977). The biting activity of *Aedes sollicitans* in New Jersey. *Mosquito News*, *37*(4), 721-724. Link

Ebsary, B. A., & Crans, W. J. (1977). The physiological age structure of an *Aedes sollicitans* population in New Jersey. *Mosquito News (USA)*. Link

Feldlaufer, M. F., & Crans, W. J. (1979). The relative attractiveness of carbon dioxide to parous and nulliparous mosquitoes. *Journal of Medical Entomology*, *15*(2), 140-142. Link

Gloria-Soria, A., Payne, A. F., Bialosuknia, S. M., Stout, J., Mathias, N., Eastwood, G., Ciota, A. T., Kramer, L.D., & Armstrong, P. M. (2020). Vector Competence of *Aedes albopictus* populations from the Northeastern United States for Chikungunya, Dengue, and Zika viruses. *American Journal of Tropical Medicine and Hygiene*, *104(3)*,1123–30. doi: 10.4269/ajtmh.20-0874. Epub ahead of print. PMID: 33355070; PMCID: PMC7941830 Link

Graham, S. P., Hassan, H. K., Chapman, T., White, G., Guyer, C., & Unnasch, T. R. (2012). Serosurveillance of eastern equine encephalitis virus in amphibians and reptiles from Alabama, USA. *The American Journal of Tropical Medicine and Hygiene*, *86*(3), 540-544. Link

Harris, M. C., Yang, F., Jackson, D. L., Dotseth, E. J., Paulson, S. L., & Hawley, D. M. (2015) La Crosse Virus Field Detection and Vector Competence of Culex Mosquitoes. *American Journal of Tropical Medicine and Hygiene*,*93(3)*,461-467. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4559680/ Link

Haufe, W. O., & Burgess L. (1960). Design and efficiency of mosquito traps based on visual response to patterns. *Canadian Entomology*, *92*,124-140. Link

Heard, P. B., Zhang, M. B., & Grimstad, P.R. (1991). Laboratory transmission of Jamestown Canyon and snowshoe hare viruses (Bunyaviridae: California serogroup) by several species of mosquitoes. *Journal of the American Mosquito Control Association*, *7*(1), 94-102. Link

Hicks, C. E. (2018). The Behavioral Response of Mosquitoes to Different Snake Odors. *Honors College Thesis.* Link

Hoel, D. F., Kline, D. F., & Allan, S. A. (2009) Evaluation of six mosquito traps for collection of *Aedes albopictus* and associated mosquito species in a suburban setting in North Central Florida. *Journal of the American Mosquito Control Association*, *25*(1), 47-57. Link

Krockel, U., Rose, A., Eiras, A. E., & Geier, M. (2006). New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. *Journal of the American Mosquito Control Association*, *22*(2), 229-38. Link

Lampman, R. L., Novak, R.J. (1996). Oviposition preferences of *Culex pipiens* and *Culex restuans* for infusion-baited traps. *Journal of the American Mosquito Control Association, 12*(1), 23-32. Link

Lindsey, N. P., Staples, J. E., & Fischer, M. (2018). Eastern equine encephalitis virus in the United States, 2003–2016. *The American Journal of Tropical Medicine and Hygiene*, *98*(5), 1472-1477. Link

Loor, K. A.,& DeFoliart, G. R. (1970), Field observations on the biology of *Aedes triseriatus*. *Mosquito News 30*(1), 60-64. Link

Lorenz, L. H., Scott, T. W., Anderson, R. A., Edman, J. D., Crans, W. J., & Costa, S. D. (1990). The relationship between size and parity status of field collected *Culiseta melanura*. *Journal of the American Mosquito Control Association*, *6*(3), 433-440. Link

Mahmood, F., & Crans, W. J. (1997). Observations of multiple blood feeding in field-collected *Culiseta melanura*. *Journal of the American Mosquito Control Association* (USA). Link

Mahmood, F., & Crans, W. J. (1998). Effect of temperature on the development of *Culiseta melanura* (Diptera: Culicidae) and its impact on the amplification of eastern equine encephalomyelitis virus in birds. *Journal of Medical Entomology*, *35*(6), 1007-1012. Link

Main, A. J., & Crans, W. J. (1986). Cache Valley virus from *Aedes sollicitans* in New Jersey. *Journal of the American Mosquito Control Association*, *2*(1), 95-96. Link

Massachusetts Arboviral Surveillance and Response Plan (2020): https://www.mass.gov/doc/2020-arbovirus-surveillance-and-response-plan/download

McCardle, P. W., Webb, R. E, Norden, B. B., & Aldrich, J. R. (2004). Evaluation of five trapping systems for the surveillance of gravid mosquitoes in Prince Georges County, Maryland. *Journal of the American Mosquito Control Association*, *20*(3),254-260. Link

McLean, R. G., Crans, W. J., Caccamise, D. F., McNelly, J., Kirk, L. J., Mitchell, C. J., & Calisher, C. H. (1995). Experimental infection of wading birds with eastern equine encephalitis virus. *Journal of Wildlife Diseases*, *31*(4), 502-508. Link

Miller, B. R., DeFoliart, G. R., & Yuill, T. M. (1977). Vertical transmission of La Crosse virus (California encephalitis group): transovarial and filial infection rates in *Aedes triseriatus* (Diptera: Culicidae). *Journal of Medical Entomology*, *14*(4), 437-440. Link

Miller, T. A., Stryker, R. G., Wilkinson, R. N., & Esah, S. (1969). Notes on the use of CO2-baited CDC miniature light traps for mosquito surveillance in Thailand. *Mosquito News, 29*, 688-689. Link

Mitchell, C. J., McLean, R. G., Nasci, R. S., Crans, W. J., Smith, G. C., & Caccamise, D. F. (1993). Susceptibility parameters of *Aedes albopictus* to per oral infection with eastern equine encephalitis virus. *Journal of Medical Entomology*, *30*(1), 233-235. Link

Mukherjee, S., Moody, E. E., Lewokzco, K., Huddleston, D. B., Huang, J., Rowland, M. E., & Moncayo, A. C. (2012). Eastern equine encephalitis in Tennessee: 2002–2008. *Journal of Medical Entomology*, *49*(3), 731-738. Link

Reisen, W. K., & Pfuntner. A. R. (1987). Effectiveness of five methods for sampling adult *Culex* mosquitoes in rural and urban habitats in San Bernardino County, California. *Journal of the American Mosquito Control Association, 3*(4), 601-606. Link

Reiter, P., Jakob, W. L., Francy, D. B., & Mullenix, J. B. (1986). Evaluation of the CDC Gravid Trap for the surveillance of St. Louis encephalitis vectors in Memphis, Tennessee. *Journal of the American Mosquito Control Association, 2*, 209-211. Link

Rochlin, I., Faraji, A., Healy, K., & Andreadis, T.G. (2019). West Nile virus mosquito vectors in North America. *Journal of Medical Entomology*, *56*(6), 1475-1490. doi: 10.1093/jme/tjz146

Sardelis, M. R., Dohm, D. J. Pagac, B., Andre, R. G., & Turrell, M. J. 2002 Experimental transmission of eastern equine encephalitis by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *Journal of Medical Entomology, 39*(3), 480-484. Link

Scott, J. J., Crans, S. C., & Crans W. J. (2001) Use of an infusion-baited gravid trap to collect adult *Ochlerotatus japonicus*. *Journal of the American Mosquito Control Association, 17*(2), 142-143. Link

Slaff, M. E., & Crans, W. J. (1977). Parous rates of overwintering *Culex pipiens* in New Jersey. *Mosquito News*, *37*(1), 11-14. Link

Slaff, M., & Crans, W. J. (1982). Impounded water as a major producer of *Culex salinarius* (Diptera: Culicidae) in coastal areas of New Jersey, USA. *Journal of Medical Entomology*, *19*(2), 185-190. Link

Slaff, M., Crans, W. J., & McCuiston, L. J. (1983). Comparison of three mosquito sampling techniques in northwestern New Jersey. *Mosquito News,* 287-290*.* Link

Unlu, I., & Farajollahi A. (2012). To catch a tiger in a concrete jungle: operational challenges for trapping *Aedes albopictus* in an urban environment. *Journal of the American Mosquito Control Association*, *28*(4), 334-337.Link

Urquhart, C., Paulsen, D., Moncayo, A., & Trout Fryxell R.T. (2016). Evaluating surveillance methods for arboviral vectors of La Crosse virus and West Nile Virus of Southern Appalachia. *Journal of American Mosquito Control Association*, *32*(1), 24-33. Link

Walker, E. D., Grayson, M. A., & Edman, J. D. (1993) Isolation of Jamestown Canyon and snowshoe hare viruses (California serogroup) from *Aedes* mosquitoes in western Massachusetts. *Journal of the American Mosquito Control Association 9(2),* 131-4. Link

West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control, June 2013, https://www.cdc.gov/westnile/resources/pdfs/wnvGuidelines.pdf

West, R. G., Mathias, D. K., Day, J. F., Acevedo, C., Unnasch, T. R., & Burkett-Cadena, N. D. (2020). Seasonal changes of host use by *Culiseta melanura* (Diptera: Culicidae) in central Florida. *Journal of Medical Entomology*, *57*(5), 1627-1634. Link

Westby, K. M., Fritzen, C., Paulsen, D., Poindexter, S., & Moncayo, A. C.(2015). La Crosse encephalitis virus infection in field-collected *Aedes albopictus*, *Aedes japonicus*, and *Aedes triseriatus* in Tennessee. *Journal of the American Mosquito Control Association, 31*(3), 233-241 Link

White, G., Ottendorfer, C., Graham, S., & Unnasch, T. R. (2011). Competency of reptiles and amphibians for eastern equine encephalitis virus. *The American journal of tropical medicine and hygiene, 85*(3), 421-425. Link

Wilke ABB, Vasquez C, Medina J, Unlu I, Beier JC, Ajelli M. Presence and abundance of malaria vector species in Miami-Dade County, Florida. Malar J. 2024 Jan 18;23(1):24. doi: 10.1186/s12936-024-04847-9. PMID: 3823877. Link

Williams, G. M., & Gingrich, J. B. (2007). Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. *Journal of Vector Ecology*, *32*(2), 285-91. doi: 10.3376/1081-1710(2007)32[285:coltgt]2.0.co;2. PMID: 18260519 Link