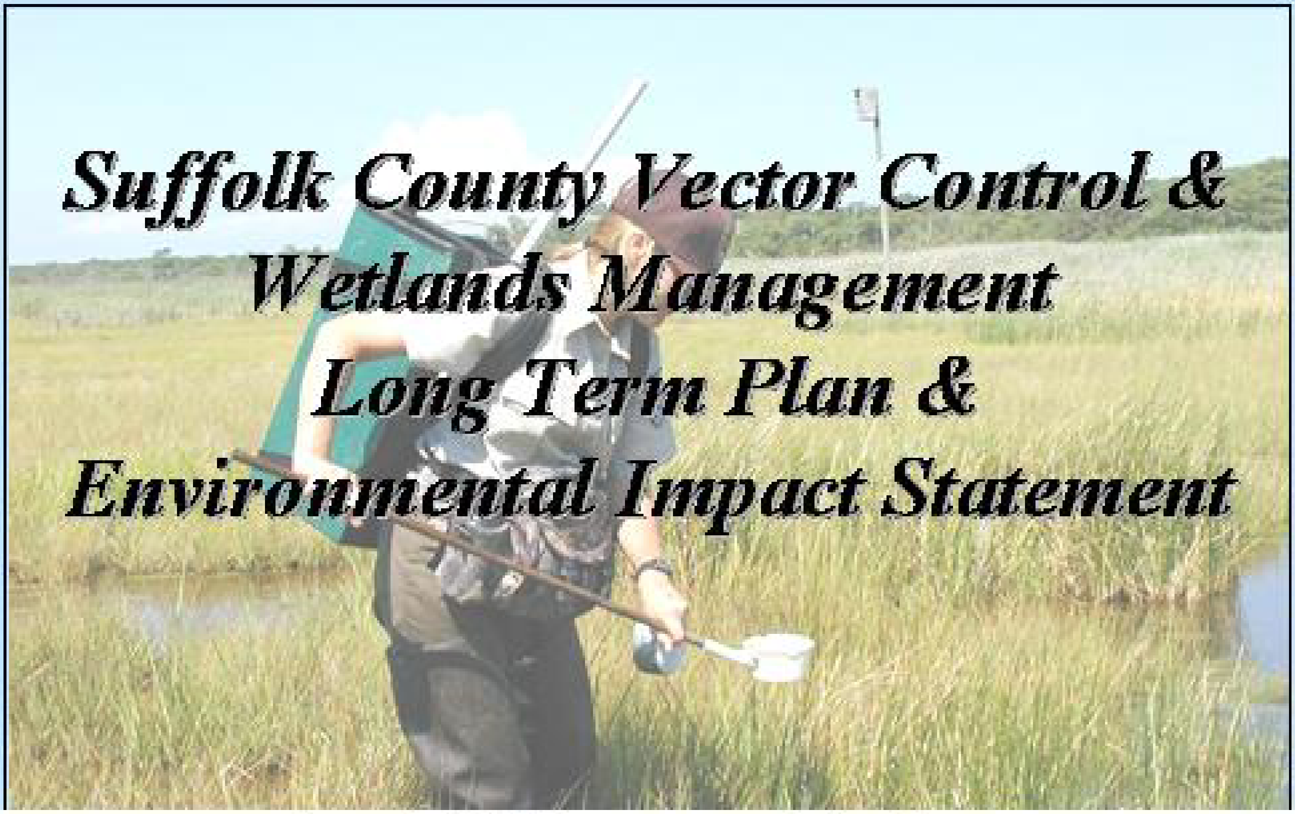

Suffolk County Vector Control & Wetlands Management Long Term Plan & Environmental Impact Statement

Task 3 Literature Review
Book 3: Mosquito Population and Disease Monitoring

Prepared for:

Suffolk County Department of Public Works
Suffolk County Department of Health Services
Suffolk County, New York

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SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS MANAGEMENT LONG - TERM PLAN AND ENVIRONMENTAL IMPACT STATEMENT

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LIST OF ABBREVIATIONS AND ACRONYMS

ABDL	Arthropod-Borne Disease Laboratory
CDC	Centers for Disease Control and Prevention
CI	Confidence Intervals
CO_2	carbon dioxide
EEE	Eastern Equine Encephalitis
GIS	Geographic Information Systems
GPS	Global Positioning Systems
MFIR	Minimum Field Infection Rate
MIR	Minimum Infection Rate
MLE	Maximum Likelihood Estimate
NYSDEC	New York State Department of Environmental Conservation
NYSDOH	New York State Department of Health
Oz	Ounce
SCDHS	Suffolk County Department of Health Services
SCVC	Suffolk County Department of Public Works, Division of Vector Control
SLE	St. Louis Encephalitis
TEA	Triethylamine
USGS	US Geological Survey
UV	ultraviolet
WEE	Western equine encephalitis
WNV	West Nile Virus

EXECUTIVE SUMMARY

The goals of a sound surveillance program include monitoring the distribution and abundance of larval and adult mosquitoes and the prevalence of mosquito-borne diseases. This is accomplished by a variety of mechanisms, including trapping mosquitoes, monitoring their breeding, analyzing them for evidence of viral activity, and monitoring other species that may have become infected by mosquito-borne disease. Results from monitoring are used in progressive mosquito control efforts to determine appropriate interventions in order to reduce human health threats and/or impermissible discomfort, minimizing the risks associated with the intervention chosen. Sufficient monitoring efforts lead to an adaptive response program, as interventions can be tailored to achieve the desired impact on the targeted mosquito populations – that is to say, effective monitoring leads to gauging the effect of interventions, and so tuning of the level of intervention to meet changing conditions.

The three most commonly used adult mosquito traps are the New Jersey light trap, the CDC light trap, and the CDC gravid trap. The New Jersey light trap attracts a variety of mosquito species and is useful for estimating changes in adult mosquito population density and species composition. Because the light trap kills mosquitoes, it cannot be used to monitor for virus activity. A number of species are not attracted to the light trap. It is also less effective for comparisons of population densities between mosquito species. Gravid traps are used to sample for egg-bearing (gravid) *Culex* spp. mosquitoes, which carry WNV. Female human biters (including *Ochlerotatus sollicitans* and *Culex* spp.) are sampled with CDC light traps to provide additional viral surveillance. The CDC light traps utilize CO_2 as an attractant.

Larval surveys can determine the location, species, and population densities of mosquito larvae for predictions of adult emergence and for gauging required control measures. They are also utilized to assess the effectiveness of adult control measures.

Another critical component of a sound monitoring program for the Long Island region is surveillance for the presence of Eastern Equine Encephalitis and West Nile Virus. CDC light traps are used at locations chosen for their history of viral activity to trap the mosquito vectors of these diseases. Sentinel chickens are used by some vector control agencies as indicators of viral activity. Serology is performed by placing chickens in an area over an extended period of time and testing their blood for the presence of antibodies to mosquito-borne diseases.

Some vector control agencies routinely trap mosquitoes in stormwater catch basins, which can be important breeding locations. Special catch basin traps are used along with CDC light and gravid traps to sample for virus activity. In addition, technological innovations for progressive control programs include the use of Global Positioning Systems (GPS) for locating key sites and improving control targeting, and Geographic Information Systems (GIS) for surveillance location mapping and data management.

Intensive disease and mosquito population monitoring is a key component of the New York State Department of Health (NYSDOH) mosquito monitoring and control guidelines. The guidelines define five risk categories by the "probability of human outbreak." The NYSDOH recommended response emphasizes increased surveillance, larval control, source reduction, and public education with each increase in the risk category. NYSDOH suggests that adulticiding targeted at areas of potential human risk be "considered" when the probability of human outbreak increases from "possible" to "probable" and only if the risk is "likely to continue to increase and bridge vectors are abundant."

1. Mosquito Surveillance

According to the Centers for Disease Control and Prevention (CDC) publication, *Guidelines for Arbovirus Surveillance Programs in the United States* (Moore *et. al.*, 1993):

> "Mosquito surveillance should have two basic activities,
>
> 1) Identifying and mapping larval habitats and
> 2) Monitoring adult activity."

The third component of all modern mosquito control monitoring programs is viral surveillance. The CDC publication identifies the goals of a "proactive arbovirus surveillance system" stating:

> "Mapping and monitoring larval habitats gives early estimates of future adult densities, and under some conditions, provides the information necessary to eliminate mosquitoes at the source. Monitoring species, density, age structure, and virus infection rates in adults provides critical early, predictive data for the surveillance system."

1.1. Larval Surveillance

Most vector control programs sample a range of aquatic habitats for the presence of pest and vector species of mosquitoes during their developmental stages. Surveys of immature mosquitoes are important aspects of an effective mosquito surveillance and control program. Larval surveillance is a critical first step in a successful mosquito control program. They are critical tools for predicting the emergence of adults and for determining the timing of larval control measures. Source reduction efforts and wetland management programs are based on the results of larval surveillance efforts. Good larval surveillance makes possible larval control with 'biorational' pesticides like Bti and growth regulators. Use of these pesticides may be more desirable than use of adulticides as they are more species specific and can be more accurately targeted spatially than pesticides used for adult mosquito control. Thorough larval surveillance can therefore reduce the need for broad scale applications of adulticides. Larval surveillance can also be used to determine the effectiveness of chemical and biological control measures by measuring the subsequent population (O'Malley, 1989).

Reed and Husband (1969) listed the advantages of conducting larval surveillance as:

- Routine larval surveillance provides a more complete and accurate record of sources of mosquito breeding, thereby providing documentation of mosquito production as a basis for treatment.
- Ongoing larval surveillance allows for continuous evaluation of insecticide application and control results.
- A clear understanding of species distribution, density, and seasonal occurrence is facilitated through routine larval surveillance.
- Routine larval surveillance enhances the knowledge provided by adult mosquito surveillance (e.g., light traps, bite counts, landing rates).
- A system for detection of insecticide resistance is provided through a larval surveillance program.

Some vector control agencies collect and identify samples from all sites inspected that contain larval mosquitoes. Such thorough larval surveillance is used to justify pesticide applications and evaluate its efficacy. Samples should be collected and identified from representative areas throughout the season, as species composition can change (O'Malley, 1989). As identification efforts can require substantial staff hours, larvae can be preserved and identification done during the winter. Routine larval collections can lead to lower use of pesticides, as only those areas found to have pest mosquitoes in sufficient numbers will warrant pesticide treatment. If only non-pest mosquitoes are identified in an area, treatment with pesticides would not be required.

It is important to organize the known breeding sites into some type of system, in the form of a route book, or preferably a Geographic Information System (GIS) map inventory. The basic tools required for larval surveillance include:

- A standard, white 400 ml-capacity dipper;
- A small pipette or eyedropper;
- A pair of boots,
- Vials,
- 6 ounce (oz.) plastic bags or some other form of container for collecting larvae;
- Labels for the collections; and
- A pencil.

Other, more specialized tools may be necessary for sampling larval habitats inaccessible to a dipper.

1.1.1. Techniques for Larval Surveillance

Mosquito larvae are found in a great variety of habitats. This fact has created a need to develop a number of different sampling techniques to ascertain the presence or absence of immature mosquitoes, and to estimate their numbers. When preparing to sample mosquito larvae it is necessary to proceed carefully, as heavy footfalls create vibrations that disturb larvae and cause them to dive to the bottom of the water. Approaching the sampling area with the sun in one's face prevents shadows, which also disturb larvae and causing them to dive to the bottom. If the wind is significant that particular day, dipping should be done on the windward side of the habitat where larvae and pupae will be most heavily concentrated. Mosquito larvae are usually found where surface vegetation or debris is present. In larger pools and ponds, they are usually confined to the margins and will not be found in open, deep water. Dipping should be done around floating debris, aquatic vegetation, logs, and tree stumps, and perimeter grasses. All individual microhabitats should be sampled. If only one microhabitat exists, then several stations should be sampled (O'Malley, 1989).

Following are eight techniques recommended by the New Jersey Mosquito Control Association for sampling of mosquito larvae and pupae with the standard pint dipper (O'Malley, 1995).

1. **The Shallow skim -** *Anopheles* larvae are normally found at the surface of the water among aquatic vegetation or floating debris. They can be collected with a shallow, skimming stroke along the surface, with one side of the dipper pressed just below the surface. Each stroke is ended before the dipper is filled, to prevent overflowing.
2. **Partial submersion** - around emergent vegetation, logs, and tree stumps, larvae may be drawn into the dipper by submerging the one edge so that the water flows rapidly into the dipper. In this method, the dipper is kept stationary in the water.
3. **Complete submersion** - Certain Culicine larvae (such as species of *Aedes* and *Psorophora*) are very active and usually dive below the surface of the water when disturbed. Here, a quick plunge of the dipper below the surface is required, bringing the dipper back up through the submerged larvae. The dipper is brought back up carefully to avoid losing the larvae with overflow current.
4. **Dipper as a background -** This is an especially useful technique in woodland pools, for early season species. The dipper is completely submerged within the woodland pool, going down into the bottom litter if necessary. A white dipper is used as a background against which larvae and pupae can be spotted. It is used to come up underneath the larvae with the dipper.

5. **"Flow in" method** - This method is useful in situations where the water is shallow, with mud, leaf litter or other debris on the substrate. Specimens can be collected by pushing the dipper down into the material on the bottom and letting the shadow surface water and mosquito larvae flow directly into the dipper.
6. **Scraping** - This method is used in permanent or semi-permanent habitats containing clumps of vegetation, such as tussocks. Water is dipped in, towards the tussock, and ended by using the dipper to scrape up against the base of the vegetation to dislodge any larvae present.
7. **Simple scoop** - This is the technique that seems to be most commonly used by field personnel for larval surveillance and is the one referred to in a lot of literature as "the standard dipping procedure." The technique involves simply scooping a dipperful of water out of a habitat. It is useful in a wide variety of habitats, especially for collecting *Culex* mosquitoes.
8. **Salt marsh** - In the case of salt marsh potholes, dips are taken in a number of spots around the edge of the pothole, dipping in toward the edge. The middle of the pothole is sampled, using either a skimming or a scooping stroke. In areas containing numerous potholes, several samples should be taken. The same combination of techniques is used to sample a salt marsh pan.

Table 1-2 lists mosquito species that are *not* routinely collected by using a dipper.

Table 1-1- Mosquito larvae that are *not* routinely collected with the dipper

Species	**Habitat**
Aedes albopictus	Tires
Anopheles barberi	Tree holes, tires, containers
Coquillettidia perturbans	Permanent water with emergent vegetation
Culiseta melanura	Cedar and red maple swamps, occasionally tires
Ochlerotatus altropalpus	Rock pools, tires
Ochlerotatus japonicus	Rock pools, tree holes
Ochleratatus triseriatus	Tree holes, tires, containers
Orthopodomyia alba	Tree holes, tires, containers
Orthopodomyia signifera	Tree holes, tires, containers
Toxorhynchites rutilus septentrionalis	Tree holes, tires, containers
Wyeomyia smithii	Pitcher plants

From O'Malley (1989)

When sampling *Wyeomyia smithii*, O'Malley (1989) recommends using either a meat baster or a pipette to remove water and larvae from the plant leaves. She describes surveillance techniques for *Coquillettidia perturbans* larvae as "pulling up emergent plants and shaking the roots into a bucket or separate cylinder." As she points out, "this is not generally satisfactory for surveillance because it requires pulling up plant species that are not always easy to dislodge from the substrate, or taking large volumes of material back to a lab for sorting." According to

Walker and Crans (1986), sampling in these environments requires use of a modified bilge pump used with a soil-sampling sieve. The bilge pump is modified into a type of syringe by removing the intake valve. To sample for larvae, the end of the bilge pump is placed into the water at the base of a cattail or other host plant. The pump is lowered into the root mat of the plant (but not into the mud), water is drawn up, and larvae discharged into a sieve."

A hospital syringe can be used when the dipper does not work. The syringe can be used to access narrow pools that cannot be accessed by the standard dipper (O'Malley, 1989). The Salem County Mosquito Extermination Commission uses a syringe fitted with surgical tubing, to withdraw larval samples from between the cracks of the dried out sediments of dredged spoil deposition sites (Kent *et.al.*, 1987). In their procedure, the water and larvae are drawn into a graduated syringe to determine if larvae are present.

1.2. Adult Surveillance

Various mosquito traps are used to monitor adult mosquitoes. Mosquito traps are used to assess the size of mosquito populations and the distribution of mosquito-borne viruses. Where the goal is an estimate of species abundance, the mosquitoes are generally trapped and killed. Traps designed for viral assessments frequently use nets and are meant to keep the mosquitoes alive.

All mosquito traps utilize some sort of attractant to lure the host-seeking female mosquitoes to a capture or killing device. Attractants include light, heat, carbon dioxide (CO_2), and chemicals such as 1-Octen-3-ol. Trap attractants mimic the exhalations, scents, and body heat released by mammals. The majority of the traps use CO_2 as the lure, as it is an easy to produce and highly effective attractant. A plume of CO_2 attracts specifically blood-feeding insects and not the extraneous insects that can make evaluation more difficult and time-consuming. Sorting can become time-consuming when species such as moths add to the trap volume and the appearance of other species increases the amount of sorting required. Other insects such as beetles stay alive in the trap and can damage mosquitoes making identification difficult. The CO_2 is often mixed with 1-Octen-3-ol, a derivative of gases produced in the rumen of cows. The addition of this gas increases the attractiveness of the trap exponentially. Following is a description of the most commonly used traps.

1.2.1. New Jersey Light Trap

The New Jersey light trap has become the standard for sampling adult mosquitoes across the nation. Mulhern developed the trap design in 1939 at the New Jersey Agricultural Experiment Station (Mulhern, 1942). It is most effective in rural areas where there are few competing light sources. Adult mosquitoes are attracted to the all-metal trap by a 25-watt incandescent light bulb mounted beneath a wide conical top. Mosquitoes attracted to the light are drawn into the trap by a downward blowing fan through a screened funnel into a quart killing-jar containing an adulticide. A clear 25-watt bulb is most widely used for consistent and comparable results. Although greater wattage bulbs will attract more mosquitoes, changing the wattage would make population comparisons between years and regions impossible.

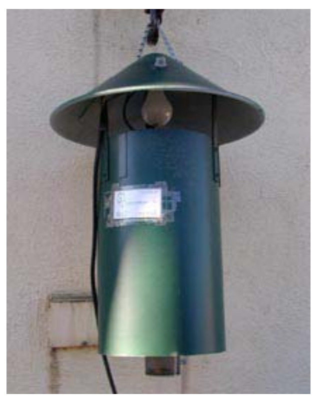

Figure 1-1 - New Jersey light trap
Source: Los Angeles County West Vector Control District

Mosquito species that can be accurately monitored by the New Jersey light trap and those that cannot are provided by Reinert (1989) and are listed in Table 1-2. The table most be properly interpreted, however, as it primarily compares species that are readily attracted to light versus those that are not. Some species such as *Ae. albopictus* and *Ochlerotatus triseriatus* are day biters and are inactive at night when the trap is running. Wayne Crans (Rutgers University, personal communication, 2004) stated, "all of these species can be monitored with the light trap but the data sets do not give relative numbers."

Table 1-2 - Light trap effectiveness with different mosquito species

Can Be Monitored	Cannot Be Monitored
o *Aedes vexans*	o *Aedes albopictus*
o *Anopheles bradleyi*	o *Anopheles punctipennis*
o *Coquillettidia perturbans*	o *Anopheles quadrimaculatus*
o *Culex pipiens*	o *Culex restuans*
o *Culex salinarius*	o *Culex territans*
o *Ochlerotatus cantator*	o *Culiseta melanura*
o *Ochlerotatus sollicitans*	o *Ochlerotatus canadensis*
o *Ochlerotatus taeniorhynchus*	o *Ochlerotatus excrucians*
o *Ochlerotatus trivittatus*	o *Ochlerotatus stimulans*
o *Psorophora columbiae*	o *Ochlerotatus triseriatus*
	o *Psorophora ferox*
	o *Psorophora ciliata*

From Reinert (1989)

Light traps should be placed in the same location year after year to best assess long-term population trends (Reinert, 1989). Reinert recommends that traps be placed along the edge of open areas close to trees and shrubs. He suggests that they be located away from buildings, windows, and exhaust vents and far from artificial illumination such as spotlights and streetlights. The traps should be protected from prevailing winds, which would inhibit the mosquito's ability to fly into it. Ideally, Reinert recommends that the trap be positioned so that the bottom of the roof of the trap is 5 ½ feet from the ground.

Although Reinert (1989) states that "little variation has been shown between trap color and species attractiveness," he cites Mulhern's (1942) suggestion that traps be painted green to blend into their surroundings. Most mosquito control agencies have adopted this practice. The underside of the trap roof is most commonly painted white to maintain a more constant trap light intensity, thus reducing sampling variability.

New Jersey light traps are best operated from May through October. May collections are usually less frequent (as nights are still too cool for mosquitoes to be active), but can document the presence of early season mosquitoes. New Jersey light traps are generally operated seven nights of the week. Some units are run four nights of the week if collections are used only to assess annual fluctuations. During the active mosquito season, mosquitoes should be collected from the traps at least three times a week to make sure that population data and disease assessments are current (Reinert, 1989). Collections should be taken more frequently if traps are the only source of adult surveillance or if they are used to determine if pesticide applications are required.

Collection of male mosquitoes can be a reliable indication of the emergence of females to follow for species with desiccation resistant eggs. Collection of a large number of male mosquitoes is an indication that a brood of females is about to hatch in the area, as male mosquitoes develop more quickly. This is not the case, however, for permanent water mosquitoes that deposit their eggs continuously. Those species do not exhibit synchronous hatches or emergences.

The New Jersey light trap will continue to be an important tool for mosquito monitoring due to its reliability. When used consistently, the trap can effectively monitor population changes for some mosquito species. It is less effective when used to compare population densities between mosquito species.

1.2.2. CDC Light Trap

The CDC light trap uses frozen CO_2 (dry ice) as an attractant. Relative to other traps, the collection of insects other than mosquitoes is significantly reduced (McNelly, 1989). The CDC light trap collects many more mosquito species and individuals than the New Jersey light trap. Unlike the New Jersey light trap, which kills mosquitoes, the CDC light trap captures and retains live mosquitoes, which is a necessity for assessing viral activity.

When first developed, the CDC light trap mimicked the New Jersey light trap, attracting mosquitoes with a white light and capturing them with a down draft from a fan. The CDC advanced the development of mosquito traps with lightweight materials including a six-volt battery and a live capture net that weighs less than two pounds in total. Because of its light weight and the capture of live mosquitoes, the CDC light trap became the standard for collection of arbovirus samples. In 1966, the CDC light trap was made even more effective with the addition of the CO_2 attractant in the form of dry ice.

Researchers believe that the full potential of the CDC light trap is not reached unless dry ice is used. The original design incorporated a much lower wattage light bulb than the 25-watt bulb used by the New Jersey light trap. The CDC light trap used with dry ice and without the light source eliminates capture of bycatch (beetles, moths, and others) that instinctively fly to light, and so simplifies identification of captured mosquito species. The trap is also less noticeable without the light, which reduces the likelihood of vandalism or theft. The rate of release of CO_2

from the trap can be controlled. A five-pound block of dry ice releases 400-500 ml of CO_2 per minute (McNelly, 1989), enough to encompass the regular "dusk to dawn" trapping period. This rate is comparable to the amount released by a large mammal in the wild (Morris and DeFoliart, 1969).

Because mosquitoes are captured live, CDC light traps are especially useful in areas where viral activity is suspected. CDC light traps are useful in areas where the death of a horse may be linked to eastern equine encephalitis (EEE) or in areas where dead birds suggest the presence of WNV. Sudia and Chamberlain (1967) prepared a review of the proper protocol for handling collections for virus isolation. The publication provides techniques for locating CDC light traps, their management, and data collection. These traps are frequently placed in "transition zones" along the edge of the dominant vegetation ecosystems. Traps in these zones are more likely to capture a wide range of mosquito species.

The New Jersey Mosquito Control Association established the following guidelines for use of the CDC light trap to help reduce sampling variability. It recommends use of 4-5 pounds of dry ice suspended adjacent to and slightly below the aluminum lid of the CDC light trap to draw the mosquitoes as close to the collection fan as possible. A photoswitch is available to automatically turn the trap on and off with daylight. An air-actuated gate system is usually recommended, especially when the trap is operated by a photoswitch. The gate stays open allowing mosquitoes to enter as long as the trap is running, but closes to prevent specimens from escaping when the trap stops running. It protects against trap failure, improperly charged batteries, or late trap pick up. This system is ideal when a live collection net is used since it offers a measure of safety against trap malfunction (McNelly, 1989). However, traps equipped with gates may be fragile and often stick open or closed (W. Crans, Rutgers University, personal communication, 2004).

Traps should be operational at least one hour before dusk until one hour after dawn, during the primary host-seeking periods for most species. They should be hung five to six feet above the ground unless specific information is needed on "canopy dwellers." For most nuisance species, this height provides a reliable indication of activity. The traps should also be set along the edges of habitats to increase trapping efficiency. Traps located between ecosystems can attract species found in both. In most situations, two traps are set out at each site to allow for equipment malfunction and system disturbances. The CDC light trap is best used for special surveillance and short periods. The New Jersey Mosquito Control Association reports that these portable, battery operated traps are:

> "Versatile and flexible and can be used effectively for surveillance studies, checking homeowner complaints, assessing the efficacy of an adulticide, or for virus surveillance."

Since the development of the original CDC light trap, a number of variations have become available. The John W. Hock Company, for example, sells several varieties. Some have been miniaturized, equipped with battery packs, and outfitted with timed switches to control the release of CO_2. Some portable trap designs have evolved from the older models that used six-volt batteries to models that run on D cells to designs that now use gel cells. Some small traps use flashlight batteries. Two of the smaller CDC light traps are described below.

The CDC Miniature Light Trap (Figure 1-2) is portable and can be sampled daily for three or four days on a single battery charge when fitted with a photo switch and air-actuated gate system. The photo switch turns the trap on at dusk and off at dawn and conserves battery life. The gate system has a butterfly check valve downwind of the motor, which is opened by air from the fan and closed by counter-balancing weights (Hock Company, 2004).

Figure 1-2 - The CDC miniature light trap
From John W. Hock Company (johnwhockco.com)

The Miniature CDC Updraft Blacklight (UV) Trap (Figure 1-3) is claimed by the manufacturer (Hock Company, 2004) to catch several times more Anopheline mosquitoes than did standard CDC miniature or New Jersey light traps. These traps can be controlled with a photo switch so that the battery life is extended to two or three nights of collection.

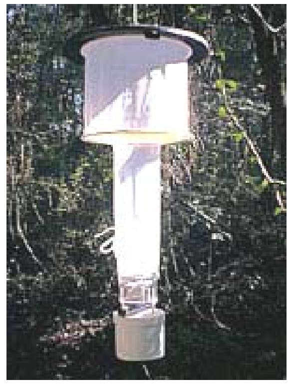

Figure 1-3 - The miniature CDC updraft blacklight trap
From John W. Hock Company (johnwhockco.com)

The manufacturer claims that blacklight (UV) attracts a greater number and diversity of mosquito species, particularly Culicoides and Phlebotomines. However, a study of electric "bugzappers" found no benefit for outdoor mosquito control for these devices that rely on UV light. Frick and Tallamy (1996) found that the bugzappers, which use ultraviolet light, do not attract mosquitoes.

1.2.3. ABC Trap

The ABC trap, manufactured by American Biophysics Corporation, is similar in design and function to the CDC light trap (McNelly, 1995). Traps based on the ABC design are widely used by vector control agencies. Its major innovation over the CDC light trap is the cooler built into the lid that holds the dry ice (Figure 1-4). The ability to hold dry ice in an insulated container extends the time required for the CO_2 to sublimate (convert from a solid to a gas). The longer the trap exudes CO_2, the longer it retains its enhanced ability to trap mosquitoes.

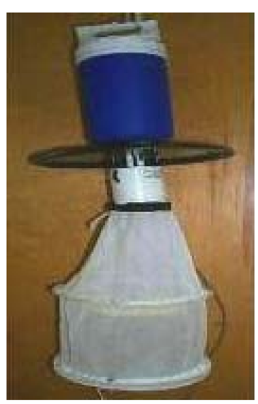

Figure 1-4 - ABC trap
Source: Hunterdon County Mosquito and Vector Control Program

1.2.4. EVS Trap

Encephalitis Virus Surveillance (EVS) traps (Figure 1-5) are used primarily to localize the presence of encephalitis or WNV. The trap uses dry ice (CO_2) as a bait to attract host-seeking female mosquitoes. The traps are typically set overnight once a week between the early spring and late summer to capture known EEE and WNV vectors.

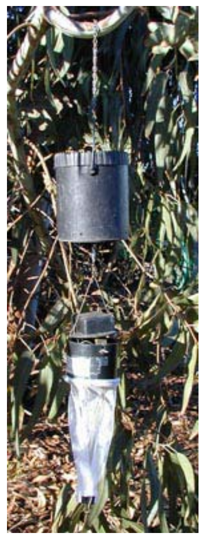

Figure 1-5 - EVS trap

Source: Los Angeles County West Vector Control District

1.2.5. CDC Gravid Trap

Paul Reiter of CDC developed the CDC gravid trap (Reiter, 1983), which selectively samples gravid (ready to deposit eggs) female house mosquitoes (*Culex quinquefasciatus*) that are seeking suitable oviposition sites. Gravid traps are also frequently used to monitor the ovipositing segment of *Cx. pipiens*, *Cx. restuans*, and *Oc. japonicus* populations (Scott *et. al*., 2000; Centers for Disease Control, 2003).

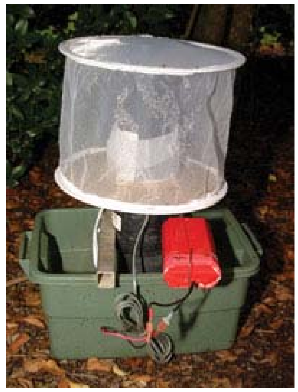

Figure 1-6 - CDC gravid trap
Source: Perich and Gleiser, 2003

The gravid trap incorporates three components:

- A base reservoir filled with oviposition materials (such as hay or manure infusion) to attract the females
- A vertically directed suction apparatus
- A top mounted collection carton.

The intake orifice of the suction apparatus is positioned one inch above the surface of the oviposition attractant. Gravid females attracted by the infusion descend into the base reservoir where they are swept into the suction apparatus and directed upward into the collection carton. The trap can be outfitted with batteries to supply power for a few nights of operation.

New research shows that blocks of expanded polystyrene floats can be used in a variety of habitats to collect ovipositing species such as the Asian Tiger mosquito (*Ae. albopictus*) and Asian Bush mosquito (*Oc. japonicus*), *Oc. triseriatus* and *Oc. hendersoni* (Scott and Crans, 2003). The white color contrasts with the dark eggs and permits a quick assessment of the presence of eggs in the field. The EPS floats are durable and can be attached to monofilament line to tether them in hard-to-reach locations. The authors concluded that the EPA float is less expensive, smaller, lighter weight, more easily transported and easier to use than other egg collection methods.

1.2.6. Resting Traps

Some vector control districts utilize resting traps or 'stations' (Louisiana Ag Center, 2003). These resting stations can be natural or man-made. They are all sheltered enclosures and include such items as rotted tree stumps, culverts, and resting boxes (open-ended red/black painted wooded boxes), from which mosquitoes are collected by aspiration. Resting stations may be used to monitor for selected adult mosquito species such as *Culiseta melanura*, a vector for EEE, and *Anopheles* species, which are vectors of malaria and other arboviruses. Crans (1995) found that the resting box was highly selective for *Culiseta melanura* and *Anopheles quadrimaculatus*, which accounted for 66.4 and 22.5 percent respectively of the 21 mosquito species collected in resting boxes in southern New Jersey. Crans describes the placement of resting boxes and the collection of mosquitoes from them. He found that they are best if placed in open shaded areas of forested habitats with high canopies that are visible to mosquitoes seeking resting places. Crans identified mature red maple swamps as ideal locations for resting boxes, yielding high numbers of mosquitoes, given the habitat's canopy and lack of ground cover. They found that mosquitoes entered the boxes in greatest numbers in the early mornings and therefore recommended making collections between 10:00 AM and 2:00 PM. Crans presents detailed collection protocols in the publication.

1.2.7. Propane Powered Traps

Propane powered mosquito collection devices may have a role in adult mosquito surveillance. Commercially available traps such as the Mosquito Magnet™ and the Mosquito Delecto™ rely on the use of heat and CO_2 to attract mosquitoes. The devices operate without the need for batteries or external power. Carbon dioxide is catalytically produced by converting propane to CO_2, water vapor, and heat. Mosquitoes attracted to the CO_2 and heat (and the optional octenol attractant) fly into a tube where they are sucked into a collection bag. A thermoelectric generator uses the excess heat from the propane combustion process to generate electricity to run the trap's fan.

These traps are very successful in trapping mosquitoes, though their use as personal protection devices may be questionable. According to Wayne Crans (Rutgers University, personal communication, 2004), a number of mosquito control agencies in New Jersey purchased these machines and discovered that they can be used as a surveillance tool. The devices attract a broader range of species than do light traps and they operate continuously rather than only during hours of darkness. Crans said that Mosquito Magnets are being used in New Jersey to sample mosquitoes on horse farms. Crans feels that the biggest drawbacks to using these devices as surveillance tools are their price and the manpower it takes to sort through the "huge" collections.

1.2.8. Bite Counts and Landing Rates

Many mosquito control agencies use Bite Counts or Landing Rates as part of their adult surveillance programs. Coastal areas use Landing Rates as part of their *Oc. sollicitans* monitoring program. This type of surveillance is useful when responding to complaints. Schmidt (1989) surveyed mosquito control agencies in New Jersey to discover a large variation in the methods used to derive these data. He differentiates the two terms defining landing rates as "the number of mosquitoes that land on an observer over a designated period of time." He recommends that landing rates be taken over a 1 or 5 minute period, depending on the density of mosquitoes (shortening the interval if the landing count exceeds 50 in 30 seconds). Usually landing rates do not involve speciation. Bite counts are defined by Schmidt as the "capture of each mosquito that comes to bite over a designated time period for species determination." Bite counts are more useful than landing rates when the biting population consists of mixed species. Schmidt suggests shortening the bite count period similarly to landing rates when densities are high. When mosquito densities are low, he recommends restricting bite counts to 10 minutes. Schmidt recommends the following guidelines for collecting both bite counts and landing rates:

Table 1-3 - Landing rate and bite count data collection

Wear solid clothing
Have all counters wear the same color clothing
Use no repellants or perfumes
Take counts from a standing position
Disturb area vegetation before beginning the counts
Count only mosquitoes that land within view
If work is conducted after sunset, use a red filter on any light source
Use a standard form to record information
Use whole numbers, do not indicate a number +
Collect mosquitoes for identification with an aspirator
Time counts to period of greatest activity for species being monitored

Adapted from Schmidt (1989)

1.2.9. Public Complaints

Romanowski and Huggins (1989) identify the utility of public complaints as a surveillance tool for mosquito control agencies. They speak to the opportunity to educate the public regarding potential breeding locations around the home. The authors also point to complaints as a chance to identify new breeding habitats that may be on private property and unknown to the agency. The number and location of complaints can be used to map areas that may be candidates for adulticiding and future source control efforts. Complaints can be used to confirm other surveillance methods. The authors recommend that all complaints be mapped and logged on a form that documents the following information from the caller:

- Day and time of call
- Name and address of caller
- How long person lived in area
- Time of day of mosquito problem
- Nature of mosquito problem

Romanowski and Huggins recommend that an inspector be dispatched as a follow up to most complaints. The inspector's observations should be documented in a report that includes what treatment was applied or recommended to the homeowner and whether the location was added to the agency's regular monitoring or control program.

1.3. Mosquito Identification

1.3.1. Larval Data Management

Data that should be collected with all larval samples in addition to species includes: date, location, habitat type, weather (temperature, cloud cover), larval or pupal density, and stages present. According to O'Malley (1989), larval density is usually expressed as numbers of larvae and pupae per dip. She recommends expressing larval density according to the following simple index developed by Belkin (1954):

BI = TLP/ND x BP

Where:

BI = the breeding index
TLP = the total number of larvae and pupae taken
ND = the number of dips
BP = the number of breeding places

Belkin defines a 'breeding place' as "each separate microhabitat or station within a site from which one to three positive dips are obtained."

1.3.2. Protecting Samples from Contamination

According to O'Malley (1989), samples can be ruined if collection equipment is contaminated with pesticides, which can cause larvae to die before are processed (identified). Clearly, sampling equipment must be isolated from pesticides if transported in the same vehicles that transport pesticides. Pesticides must also be kept out of laboratory or identification areas.

1.3.3. Adult Mosquito Sample Identification and Processing

Mosquitoes can be identified soon after trap collection for immediate decision making purposes or they can be held for days, weeks or months and still be accurately identified. Small vector control programs identify their light trap collections during the winter to assess population densities. Subsamples are taken when trap collection rates are exceptionally high. A subsample is generated by spreading the trap collection across a grid; generally, one quarter of the collection sampled. At least 100 mosquitoes should be identified for statistical purposes (Reinert, 1989).

Reinert (1989) suggested that light trap collections should be identified at least three times per week or more frequently if the traps are the agency's sole source of adult surveillance or if the data is used to determine pesticide applications. He emphasized the need for speed and accuracy when using these traps to guide day-to-day operations, recommending identification within 24 hours of collection. All trapped mosquitoes used for population assessments are keep cool to prevent sample deterioration.

A recent publication by the California Department of Health Services, Mosquito and Vector Control Association of California, and the University of California (California DOHS *et. al.*, 2004) describes procedures recommended for the handling and processing or live mosquitoes for identification and subsequent viral testing. The publication suggests, "Mosquitoes should be anesthetized, identified under a dissecting microscope, sorted by sex and female metabolic status, (*i.e.*, empty or unfed, blood fed, or gravid), and counted." They recommend counting females "into 10 pools of approximately 50 females per site collection date for virus monitoring." Mosquitoes can be anesthetized by subjecting them to cold temperatures, CO_2, or triethylamine (TEA). The publication recommends use of TEA as it permanently immobilizes mosquitoes with minimal mortality and no loss of virus activity (Kramer *et. al.*, 1990). According to the publication, mosquitoes kept humid and refrigerated can remain alive for in covered petri dishes for one or two days. Alternatively, mosquitoes can be frozen as long as they are sorted and processed on a refrigerated table to prevent loss of virus activity. Frozen mosquitoes may however, become brittle and parts that are necessary for identification may be lost. The California agency emphasizes the importance of removing extraneous mosquito parts and other insects prior to having the pool tested for viral activity. Samples for viral testing are placed in sealed vials (to avoid contact with CO_2) and frozen at –70ºC (in an ultralow refrigerator or using dry ice). Samples are shipped frozen with dry ice to a specialized testing laboratory (usually a state department of health or university) for viral testing.

1.3.4. Management of Light Trap Data

New Jersey light trap data should be averaged to reduce the influence of a few very high collections on all trap data from a series (Downing, 1976). Downing suggests that the Williams Mean analysis "gives a more accurate estimate of mosquito abundance from a series of traps." Reinert (1989) confirms the advisability of using Williams Mean analysis (Table 1-4) to reduce "the influence of a few very high traps on the average of all traps in the series."

When comparing year-to-year light trap data, Reinert recommends excluding the months of May, September, and October due to "widely fluctuating climatic conditions." Downing suggests the use of the five point moving mean to "reduce the influence of climatic fluctuations on the day-to-day trap collections." Reinert also supports the use of the five point moving mean to correct for nightly variations as shown below (Table 1-4). The five point (day) moving mean generates an average for five consecutive days, which moves ahead one day at a time.

Table 1-4 - Useful formulas for analyzing mosquito light trap data

Formula for Williams Mean	**Formula for Five Point Moving Mean**
[EXP ({LOG (tl + 1) + LOG (t2 + 1)...}/n)] - 1 Where: t = each trap collection n = number of traps	1st point = (day 1 + day 2 + day 3 + day 4 + day 5)/5 2nd point = (day 2 + day 3 + day 4 + day 5 + day 6)/5 3rd point = (day 3 + day 4 + day 5 + day 6 + day 7)/5 etc ...

2. Arbovirus Surveillance

More than 500 arboviruses (arthropod-borne viruses) are transmitted by mosquitoes world-wide (Eldridge and Edman, 2000). In Suffolk County, viral surveillance is directed primarily at two pathogens, EEE and WNV. CDC light and gravid traps are placed on weekly basis at thirty or more locations. Trapping sites are chosen based on their history of viral activity and the presence of arboviruses in birds, horses, and humans. In order to locate virus in these organisms, SCDHS has a Dead Bird Hotline (June through September), consultant veterinarians, and active human surveillance. From mosquito surveillance, the Suffolk County Arthropod-Borne Disease Laboratory (ABDL) has processed an average of 44,000 live, adult mosquitoes annually for EEE and WNV analysis over the period 2000 to 2004. The mosquitoes are sorted by species, frozen, and sent to Albany. The actual analysis is conducted by the New York State Department of Health (NYSDOH) Virology Laboratory because of the biohazard involved in testing for EEE and WNV. The SCDHS has recently begun using the RAMP rapid tests for WNV detection (see section 2.6.2, below). The NYSDOH, New York State Department of Environmental Conservation (NYSDEC), and CDC monitor other WNV indicators such as unusual bird deaths. NYSDOH will also monitor hospitals and reach out to physicians to quickly detect human cases. Arbovirus surveillance is necessary for SCVC, in cooperation with SCDHS and NYSDOH, to gauge the potential for disease transmission and take appropriate action.

2.1. Sentinel Chicken Surveillance

Sentinel chicken serology is performed by placing chickens in an area over an extended period of time and testing their blood for the presence of antibodies to WNV and EEE. For example, the Los Angeles County West Vector Control District maintains and operates 15 flocks of chickens for this purpose placed strategically throughout its District. The chickens are bled once every two weeks during the months of May through October. Blood samples are processed and tested in the District's laboratory. Although the tests could be conducted by an independent laboratory, performing these tests in house results in much quicker identification of viral activity. Only a very small amount of blood is required from each chicken every other week. According to the Los Angeles County West Vector Control District, the chickens represent "a critical element of the District's surveillance program and help to prevent any transmission of SLE and Western equine encephalitis (WEE) to the human population" (Los Angeles County West, 2001).

Suffolk County Vector Control utilized sentinel chickens in 2000. According to SCVC director, Dominick Ninivaggi (personal communication, 2004), "for reasons no one has been able to explain, [the chickens] had no seroconversions, even among chickens maintained in areas where WNV was repeatedly found in mosquitoes." Ninivaggi explained that the County's experience "is similar to that of others in the northeast, where few chickens convert." Wayne Crans suggested (W. Crans, Rutgers University, personal communication, 2004) that the reason for this may be associated with the particular mosquito species involved. He suggests that California and Florida sentinel chicken programs are monitoring mosquito species that readily feed on caged chickens such as *Cx. tarsalis*, the WEE, WNV, and SLE vector in California. Similarly, *Cx. nigripalpus* is the SLE and WNV vector in Florida. *Cx. pipiens*, is the northeastern WNV vector. According to unpublished work cited by Crans, it has difficulty feeding on birds that exhibit defensive behavior.

2.2. Sentinel Crow Surveillance

Crows have been used extensively in the northeastern United States as sentinels for WNV (Eidson *et. al.*, 2001). In 1999, the New York State Department of Health received reports of 17,339 dead birds, including 5,697 crows (33%). The authors found that "bird deaths were critical in identifying WN virus as the cause of the human outbreak and defining its geographic and temporal limits." Crows could "provide a sensitive method of detecting WN virus" if the surveillance system was established before a WNV outbreak. 89 percent of the WNV positive dead birds were American crows, though the virus was isolated from dead birds of 19 other species. There are limitations of utilizing reports of dead crows for virus surveillance, but public awareness and participation is key to the success of such a program. Media coverage can also skew public reporting of dead or ill birds. A greater number of dead birds were reported in areas with greater media coverage of WNV activity and related bird deaths. Furthermore, collecting, processing, and analyzing dead birds for WNV is time consuming and expensive. Decisions on which birds to collect and assess for WNV can then add variability to the data. Another limitation is the geographic difference in bird and human cases of WNV. Birds could have been infected in one location and flown to another where they died. Despite the limitations, Eidson *et. al.* conclude that bird deaths can give an indication of the geographic spread of the virus, particularly when WNV isolation in humans, mosquitoes, or other animals are no longer reported.

2.3. Other Sentinel Bird Species

A number of vector control agencies have an active avian surveillance program that samples populations of wild birds for the presence of arboviruses before the viruses appear in the human population. The following program description is taken primarily from the Harris County (Texas) Mosquito Control Division's Annual Summary Report (Harris County, 2001).

The capturing and handling of wild birds is controlled by federal law (Federal Migratory Treaty Act). Permits must be obtained from the US Fish and Wildlife Service and the NYSDEC. Wild birds are often captured with a Japanese Mist net (Figure 2-1). Small samples of blood sera (the fluid portion of the blood that contains antibodies) are removed from birds (Figure 2-2) and the bird banded before being released unharmed. Banding allows the agency to avoid sampling the same bird twice, unless it is specifically desired.

Harris County had 968 established avian sites in 2000. The sites are selected based on the known or presumed presence of flyways. The nets are baited with commercial bird feed, set up before sunrise, and taken down by late morning. Nets are checked every 15 minutes or less and collapsed if it rains as hypothermia would be a concern in birds captured in the rain. Captured birds are held in opaque cotton bags until processed. Blood is drawn from the jugular or wing vein. Harris County personnel record the following characteristics for captured birds:

Table 2-1 - Characteristics measured from sentinel birds

• Species • Band number • Age	• Sex • Weight • Body fat • Molting pattern

In 2000, the most frequently collected birds were the house sparrow (73.0 percent of all birds collected) and the blue jay (7.6 percent). The Harris County Health Department conducted "antibody prevalence rate" tests for St. Louis Encephalitis among the bird species testing positive to determine which had higher rates. The tests help them determine what role each species might play in the transmission of the virus. Of the nine species testing positive for the virus, the following seven are found on Long Island:

- blue jay
- Northern mockingbird
- house sparrow
- European starling
- Northern cardinal
- common grackle mourning dove
- brown-headed cowbird

All of these species have been reported by the CDC and the US Geological Survey (USGS) as dead and infected with WNV (CDC, 2004; USGS, 2004). Nationwide, as of May 2004, 234 species of birds have been reported infected by WNV. The USGS National Wildlife Health Center provides diagnostic support and research results to Federal, state, and local wildlife agencies, as well as to public health departments that are utilizing dead wild birds as sentinels for detecting the WNV (USGS, 2004). They also maintain a list of bird species that have been infected by the WNV.

Figure 2-1 - Bird captured in net for arbovirus surveillance

(From Centers for Disease Control Public Health Image Library)

Figure 2-2 – Blood sampling from captured bird

(From Centers for Disease Control Public Health Image Library)

2.4. Stormwater Catch Basin Collections

Some vector control agencies routinely trap mosquitoes in stormwater catch basins as they can be important mosquito breeding locations. The Division of Mosquito Control in Harris County, Texas sampled 2,908 stormwater catch basin traps (of its own design) in 2001 (Harris County, 2001). It also used 92 CDC light traps with a dry ice attractant and two gravid traps in association with the catch basin traps, to sample this population for virus activity. Harris County is much larger than Suffolk County. It is the third largest county in the nation, includes the city of Houston, and is home to three million residents. The major concern there is freshwater mosquitoes and the standing water in stormwater control systems is one of their prime habitats. The stormwater catch basin traps accounted for almost 90 percent of the traps in the county and over 99 percent of the female mosquitoes collected. Harris County samples the catch basins weekly, biweekly, or monthly, depending on historic viral activity. From June through September, when viral activity is highest, trapping is conducted throughout the entire county. Sampling beyond that period is conducted in areas where viral activity occurs most frequently and areas where human cases are confirmed. Ray Parsons, Director of the program, believes that intensive surveillance is key for establishing an effective control program (R. Parsons, Harris County Mosquito Control, personal communication, 2003). Such a program works well in Harris County and other locales where the catch basins hold water throughout the year. It could have some utility in portions of Suffolk County.

2.5. Site Selection

Viral surveillance in Suffolk County is directed at EEE and WNV and follows the most recent CDC and NYSDOH guidelines. Traps are placed in areas that have a history of viral activity or are locations where infected birds or other animals have been located. On an annual basis, the Suffolk County ABDL collects and processes approximately 44,000 live, adult mosquitoes for viral activity (S. Campbell, Suffolk County ABDL, personal communication, 2004).

Sampling of stormwater catch basins is important, as *Cx. pipiens* and *Cx. restuans*, the primary vectors of WNV, are found there in significant numbers. The mosquitoes are found only in those catch basins that retain standing water. Standing water will be found in catch basins if they have not been routinely maintained (and fine sediments restrict stormwater infiltration). Other catch basins in low-lying areas may have standing water from groundwater infiltration.

2.6. Virus Analysis

2.6.1. Determining Infection Rates

According to New York's 2001 WNV Guidelines (NYSDOH, 2001), the goal of virus analysis is to "determine the proportion of the mosquito population carrying the virus, or the Minimum Field Infection Rate (MFIR, expressed as the number infected per 1000 specimens tested)."

New York State's West Nile Virus Response Plan (NYSDOH, 2001) recommends that only pools of certain mosquito species should be submitted to the State laboratory for testing. For the EEE virus, *Culiseta melanura* should be tested. For WNV, *Culex* species should be tested. They include *Cx. pipiens, Cx. restuans, Cx. salinarius*, and species associated with *Cx. pipiens* (*Oc. japonicus, Oc. triseriatus*). The Plan recommends that mosquitoes should be grouped by species, sites and collection date into a group, or "pool," of 50 individual mosquitoes of the same species, that have been collected by the same method during one week of collection activities. According to the CDC, "Pooling of mosquitoes when estimating infection rates arises from the practical efficiency of combining individuals when infection rates are low" (Biggerstaff, 2003).

Although "Infection Rate" is calculated from the data, the rate is not truly an "infection rate" as used by epidemiologists, but rather the number of infected specimens per 1000 specimens tested or the sample "incidence rate." The rate (level) of infection in the mosquito population cannot be determined from this data because of the impact of random fluctuation on small frequencies. The incidence rate, however, is very useful in predicting (based on historical data) the likelihood of a disease outbreak (Biggerstaff, 2003).

The CDC has developed a method for estimating mosquito population infection rates based on the number of positive mosquito pools (Biggerstaff, 2003). The CDC publication points to the need for specialized statistical methods when pool sizes are equal or unequal. Statistical information and references are provided in the CDC publication. The following discussion is included in the documents (which refers to an included computer spreadsheet to allow for simplified computation of the referenced statistics):

> "A traditional analysis in this setting uses the minimum infection rate (MIR) to estimate the population infection rate from pooled samples. The MIR is easy to compute, and corresponding confidence intervals (CI), based on the textbook, large-sample binomial CI are also easily computed. Alternatives to the MIR include maximum likelihood estimation (MLE)…. This Excel® Add-In computes the MIR, the Maximum Likelihood Estimate (MLE) and a bias-corrected MLE for point estimation."

2.6.2. Rapid Identification Technologies

At least two rapid WNV tests are commercially available, Response Biomedical's RAMP® WNV detection assay and VecTest™ WNV assay from Medical Analysis Systems (MAS). Burkhalter *et. al.* (2003) evaluated the two systems in collaboration with CDC and Health Canada laboratories to test their sensitivity and specificity. They summarized the two systems as follows:

> "While both assays are based on immunochromatographic principles, each test has unique characteristics that provide their own advantages and disadvantages to laboratories conducting WNV surveillance. Serially diluted stock seed WNV and field-collected positive mosquito pools were used to determine sensitivity. Specificity was determined by testing each assay with St. Louis Encephalitis (SLE) virus. The RAMP system uses test samples that have been mixed with fluorescently dyed latex particles conjugated to WNV-specific antibodies. The mixture is added to a test strip contained within a cartridge and migrates along the strip. When WNV is present in the sample, these antigen-bound particles are immobilized at the detection zone and additional control particles are immobilized at an internal control zone. The RAMP reader measures the amount of fluorescence emitted by the particles bound at each zone. Using the ratio between the two fluorescence values, the reader displays the test result in

Relative Units. Based on similar principles as the RAMP, the VecTest WNV Antigen Assay uses monoclonal antibodies bound to colloidal gold to identify the presence of WNV antigen in a sample. A VecTest dipstick is added to a sample that has been prepared in the assay's buffer. Antigen present in the sample binds to the specific antibody with a gold sol particle label."

The report reached the following conclusions:

- The results demonstrate that the RAMP assay is more sensitive than VecTest in both lab-generated virus seed samples and field-collected mosquito pools.
- Outlined below, the advantages and disadvantages of each test determine which assay best suits a laboratory's objective.

The report summarizes the advantages and disadvantages of the two systems as follows:

RAMP Advantages

- Test is simple to use and does not require laboratory or technical experience
- Approximately 100-fold more sensitive than VecTest
- Results read by the machine are objective and easy to interpret
- Portable – reader can run on battery power for approximately 100 runs and can be recharged overnight; while grinding using a vortex is highly recommended, it is not required according to the manufacturer's instructions

RAMP Disadvantages

- Requires greater sample preparation and handling than VecTest
- Time intensive – the machine reads one cartridge at a time and read time is roughly one minute per sample

VecTest Advantages

- Test is simple to use and does not require laboratory or technical experience
- Results are easy to interpret when distinct bands are present on the strip
- The rapidity of sample preparation and test performance allows a potentially higher throughput
- Portable – kit contents are easier to transport than RAMP equipment; however, VecTest requires battery power to grind mosquitoes with a vortex

VecTest Disadvantages

- Not as sensitive as RAMP
- Results read by individuals are subjective and can be difficult to interpret when band intensity is weak

2.7. Mapping and Data Management

Progressive mosquito control agencies now emphasize the collection and processing of surveillance data in timely manners that allow for program actions to be modified to reflect changing conditions (as reported by the surveillance data).

One way this is addressed is by carefully determining the efficacy of interventions. This can be done by pairing treatment and control areas, and collecting appropriate data both before and after treatment at the two sites, and thereby inferring the impact of the treatment. Alternately, surveillance data prior to treatment can be collected, and compared to data collected after treatment (within the affected zone). This approach is somewhat less effective in that factors outside of treatment are not easily assessed without some form of control site (J. Goddard, Mississippi Department of Health, personal communication, 2004).

Another approach has been instituted in New Jersey. Data from 84 New Jersey light traps are contributed by county mosquito control agencies, and are used by the state agency to calculate trends in mosquito populations for species of nuisance or health concerns. The data are collected and analyzed on a weekly basis. Calculations are made based on regional distributions, with emphases on mosquito habitat and land use. Temporal trends are developed over the course of a breeding season, and analyzed by mosquito ecologists at Rutgers University, to allow for statewide and regional evaluations of changing mosquito populations, in response to control and/or changes in habitat. This enables local control agencies to make decisions that address the problems in a well-defined context (W. Crans, Rutgers University, personal communication, 2004).

SCVC and the Suffolk County ABDL have instituted the use of GPS and GIS for surveillance location mapping and data management. The effort reduces the time required for data transcription and better targets control efforts. In addition to ongoing County data management efforts, the Long-Term Plan project includes digitization of paper records into GIS formats to generate an historic record of breeding areas, larviciding applications, wetland management efforts, and virus activity locations. This will increase the power of the GIS analyses of the current data. Andrew Spielman (Harvard School of Public Health, personal communication, 2004) notes that although GIS use is becoming more common in vector control operations, it is not clear if the appropriate data are being collected. Furthermore, although GIS can generate information that can be used to guide key management decisions (for example, comparisons of trap data across years at similar dates to determine if mosquito trends seem to require control activities), it is far from certain that mosquito managers have yet become accustomed to accessing this kind of material.

3. Mosquito Control Guidelines

Intensive disease and mosquito population monitoring is a key component of the New York State Department of Health mosquito monitoring and control guidelines published in its *West Nile Virus Response Plan* (NYSDOH, 2001). The State's guidelines are a phased approach that defines five risk categories by the "probability of human outbreak." The State guidelines are summarized in Appendix A of its report, which is reproduced in Table 3-1. The "recommended response" emphasizes increased surveillance, larval control, source reduction, and public education with each increase in the risk category. NYSDOH suggests that adulticiding targeted at areas of potential human risk be "considered" when the probability of human outbreak increases from "possible" to "probable" and only if the risk is "likely to continue to increase and bridge vectors are abundant." The State guidelines qualify the need for adulticiding with the following caveats:

> "In general, the finding of a WNV positive bird or mosquito pool does not by itself constitute evidence of an imminent threat to human health and warrant mosquito adulticiding. Adulticiding should be considered only after careful consideration of the WNV risk to human health by taking into account multiple factors, including documentation of the presence of West Nile Virus in the area, the numbers and species of the vector populations, the vectors' physiologic age, the density and proximity of human populations, the time of year, weather conditions, physiography of and accessibility to the area where the vector is located, rapidity of response required as determined by the seriousness of the public health threat, potential impact on people and the environment, and the likelihood that vectors in nearby areas not subject to control measures will migrate from the area if not subject to control. In general, ground application of pesticides should be the preferred method of control. Aerial spraying should be used only when necessary because of geographic considerations and should be limited to the immediate area where the vector population has been documented to exist through vector surveillance and to adjacent areas considered at risk for imminent disease transmission."

The New York State guidelines recommend the implementation or intensification of adulticiding at the highest risk category when an "outbreak is in progress" defined as multiple confirmed cases of WNV in humans and conditions favoring continued transmissions to humans. Some take issue with this approach. They argue that early intervention is needed to prevent the onset of human cases. Wayne Crans stated that: "

> During the 1999 outbreak of WNV in New York City, the control measures applied to the adult mosquito population after human cases became evident probably did little to change the outcome because the transmission cycle had already peaked and the disease was already on the downswing. NYC kept applying adulticides even though most of the *Culex* were already in hibernaculae"
>
> (W. Crans, Rutgers University, personal communication, 2004).

The main objective of adult mosquito control as defined in the State guidelines is "to decrease the risk of a human outbreak of West Nile virus (WNV) infections." The NYSDOH recommends that this be accomplished by:

- Continuing to stress reduction in mosquito habitats;
- Larviciding where feasible and practical;
- Using personal mosquito protection measures, especially for the elderly and immunocompromised.

The NYSDOH stresses that "adulticiding is supplementary to these measures and is a local decision" that should be based on considerations listed in their guidelines. The NYSDOH guidelines make the following statement concerning triggers for spraying:

> "Adulticiding should be considered only when there is evidence of WNV epizootic activity at a level suggesting high risk of human infection (for example, high dead bird densities, high mosquito infection rates, multiple positive mosquito species including bridge vectors, horse or mammal cases indicating escalating epizootic transmission, or a human case with evidence of epizootic activity) and abundant adult vectors. In general, the finding of a WNV positive bird or mosquito pool does not by itself constitute evidence of an imminent threat to human health and warrant mosquito adulticiding."

The National Park Service at the Fire Island National Seashore has also developed criteria to guide their mosquito and mosquito-borne disease monitoring efforts (Ginsberg, 2002). The monitoring effort is escalated as defined thresholds are reached. The goal is to minimize control efforts (and therefore potential human and environmental impacts) by increased monitoring based on evidence of arboviral infection (EEE and WNV). As with the NYSDOH guidelines and SCDHS practices, the Park Service considers intervention if "conditions strongly suggest disease risk to humans" or if the "risk of disease transmission would be substantially lowered by the intervention."

Table 3-1 - New York State suggested guidelines for phased response to West Nile Virus surveillance data

Risk Category	Probability of Human Outbreak	Definition	Recommended Response
0	None	Off-season; adult vectors inactive; climate unsuitable.	Develop WNV response plan. Secure surveillance and control resources necessary to enable emergency response. Initiate community outreach and public education programs
1	Remote	Active mosquito season; areas anticipating WNV epizootic in 2001; no identified surveillance findings indicating WNV epizootic activity.	Response as in Category 0, plus: Conduct entomologic survey (inventory and map mosquito populations); source reduction; use larvicides at specific sources identified by entomologic survey and targeted at likely amplifying and bridge vector species; maintain vector and virus surveillance; community outreach and public education, emphasizing source reduction; avian mortality and human encephalitis/meningitis surveillance.
2	Possible	Active mosquito season; areas with confirmation of WNV activity in birds and/or mosquitoes.	Response as in Category 1, plus: increase larval control and source reduction and public education emphasizing personal protection measures, particularly among the elderly. Conduct active human surveillance and other surveillance activities to further quantify epizootic activity (e.g., mosquito trapping and testing).
3	Probable	Active mosquito season; quantitative measures indicating WNV epizootic activity at a level suggesting high risk of human infection (for example, high dead bird densities, high mosquito infection rates, multiple positive mosquito species including bridge vectors, horse or mammal cases indicating escalating epizootic transmission, or a human case with evidence of epizootic activity) and abundant adult vectors.	Response as in Category 2, plus: expand public information program to include TV, radio, and newspapers (use of repellents, personal protection, continued source reduction, risk communication about adult mosquito control); continue active surveillance for human cases; consider adult mosquito control program targeted at areas of potential human risk if the risk is likely to continue to increase and bridge vectors are abundant.*
4	Outbreak in Progress	Multiple confirmed cases in humans; conditions favoring continued transmission to humans (see Category 3)	Response as in Category 3, plus: implement or intensify emergency adult mosquito control program, enhanced risk communication about adult mosquito control, monitor efficacy of spraying on target mosquito populations.

*In general, the finding of a WNV positive bird or mosquito pool does not by itself constitute evidence of an imminent threat to human health and warrant mosquito adulticiding. Adulticiding should be considered only after careful consideration of the WNV risk to human health by taking into account multiple factors, including documentation of the presence of West Nile Virus in the area, the numbers and species of the vector populations, the vectors' physiologic age, the density and proximity of human populations, the time of year, weather conditions, physiography of and accessibility to the area where the vector is located, rapidity of response required as determined by the seriousness of the public health threat, potential impact on people and the environment, and the likelihood that vectors in nearby areas not subject to control measures will migrate from the area if not subject to control. In general, ground application of pesticides should be the preferred method of control. Aerial spraying should be used only when necessary because of geographic considerations and should be limited to the immediate area where the vector population has been documented to exist through vector surveillance and to adjacent areas considered at risk for imminent disease transmission.

<u>The above table is taken directly from The New York State Department of Health West Nile Virus Response Plan, 2001</u>

REFERENCES

Belkin, JN. 1954. Simple larval and adult mosquito indexes for routine mosquito control operations. *Mosquito News* 14:127-131.

Biggerstaff, B. 2003. *PooledInfRate: a Microsoft® Excel Add-In to Compute Prevalence Estimates from Pooled Samples*. Centers for Disease Control and Prevention, Fort Collins, CO.

Burkhalter, KL, R. Lindsay, R. Anderson, A. Dibernardo, H. White, M. Drebot, W. Fong, and R. Nasci. 2003. *Evaluation of Commercial Assays for Detecting West Nile Virus Antigen*. Centers for Disease Control and Prevention, Fort Collins, CO.

California Department of Health Services, Mosquito and Vector Control Association of California, and the University of California. 2004. *California Mosquito-Borne Virus Surveillance and Response Plan*. 51 pp.

Centers for Disease Control. 2004. *Bird species reported to CDC's West Nile Virus avian mortality database from 1999-present*. Vertebrate Ecology, West Nile Virus. http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm

Centers for Disease Control. 2003. *Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control*. 3rd Revision. National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases. 80 pp.

Crans, WJ. 1995. Resting boxes as mosquito surveillance tools. *Proceedings of the New Jersey Mosquito Control Association* 82:53-57

Downing, JD. 1976. Statistical analysis and interpretation of mosquito light trap data. *Proceedings of the New Jersey Mosquito Control Association* 63:127-133.

Frick, TB and DW Tallamy. 1996. Density and diversity of nontarget insects killed by suburban electric insect traps. *Entomological News* 107(2): 77-82.

Eidson, M., N. Komar, F. Sorhage, R. Nelson, T. Talbot, F. Mostashari, R. McLean, and the West Nile Virus Avian Mortality Surveillance Group. 2001. Crow deaths as a sentinel surveillance system for West Nile Virus in the northeastern United States. *Emerging Infectious Diseases* 7(4): 615-620.

Eldridge, BF and JD Edman. 2000. *Medical Entomology*. Kluwer Academic Press, Boston and New York. 659 pp.

Ginsberg, HS. 2002. *Mosquito Surveillance and Management Protocol, Fire Island National Seashore*. USGS Patuxent Wildlife Research Center, Patuxent, MD

Harris County. 2001. *Annual Summary Report*. Mosquito Control Division. Mosquito Control Field Operations, Harris County, TX.

Hock Company. 2004. *Mosquito and Sand Fly Traps*. (www.johnwhockco.com/products /sandflytraps.htm).

Kent, RB, W. Fisher, and P. Mulligan. 1987. Surveillance and control of New Jersey mosquito populations on U.S. Corps of Engineers' dredge spoil areas: a pilot program utilizing aerial insecticides. *Proceedings of the New Jersey Mosquito Control Association* 74:78-84.

Kramer, LD, SB Presser, EJ Houk, and JL Hardy. 1990. Effect of the anesthetizing agent triethylamine on western equine encephalomyelitis and St. Lois encephalitis viral titers in mosquitoes(Diptera: Culicidae). *Journal of Medical Entomology* 27:1008-1010.

Los Angeles County West. 2001. *Mosquito-Borne Disease Surveillance - Sentinel Chicken Surveillance*. http://www.lawestvector.org/

LSU Ag Center. 2003. *Louisiana Mosquito Abatement Plan*. Louisiana State University Agricultural Center Research and Extension. http://www.lsuagcenter.com/subjects /mosquito

McNelly, JR. 1989. The CDC Trap as a special monitoring tool. *Proceedings of the New Jersey Mosquito Control Association* 76:26-33.

McNelly, JR. 1995. An introduction to the ABC trap. *Proceedings of the New Jersey Mosquito Control Association* 82: 47-52.

Moore, CG, RG McLean, CJ Mitchell, RS Nasci, TF Tsai, CH Calisher, AA Marfin, PS Moore, and DJ Gubler. 1993. *Guidelines for Arbovirus Surveillance Programs in the United States*. Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services. Fort Collins, Colorado

Morris, CD, and GR DeFoliart. 1969. A comparison of mosquito catches with miniature light traps and CO_2-baited light traps. *Mosquito News* 29(3):42.

Mulhern, TD. 1942. *New Jersey Mechanical Trap for Mosquito Surveys*. Circular 421. New Jersey Agricultural Experiment Station. Rutgers University, New Brunswick, NJ. 8 pp.

New York State Department of Health. 2001. *New York State West Nile Virus Response Plan – Guidance Document*. 97 pp.

O'Malley, C. 1989. Guidelines for larval surveillance. *Proceedings of the New Jersey Mosquito Control Association* 76:45-55.

O'Malley, C. 1995. Seven ways to a successful dipping career. *Wing Beats* 6(4): 23-24.

Perich, MJ, and RM Gleiser. 2003. Operation mosquito fights bites. *Louisiana Agriculture* 46(3):

Reed, DE, and RC Husbands. 1969. Integration of larval surveillance techniques in the operational program of Fresno Westwide Mosquito Abatement District. *Proceedings of the California Mosquito Control Association* 37:98-101.

Reinert, WC. 1989. The New Jersey light trap: an old standard for most mosquito control programs. *Proceedings of the New Jersey Mosquito Control Association* 76:17-25.

Reiter, P. 1983. A portable battery operated trap for collecting gravid *Culex* mosquitoes. *Mosquito News* 43:496-498.

Romanowski, M., and RD Huggins. 1989. Complaints: an underrated surveillance parameter. *Proceedings of the New Jersey Mosquito Control Association* 76:39-41.

Schmidt, RF. 1989. Landing rates and bite counts for nuisance evaluation. *Proceedings of the New Jersey Mosquito Control Association* 76:34-37.

Scott, JJ, SC Crans, and WJ Crans. 2000. Use of an infusion baited gravid trap to collect *Ochlerotatus japonicus*. *Journal American Mosquito Control Association* 17:142-143.

Scott, JJ, and WJ Crans. 2003. Expanded polystyrene (EPS) floats for surveillance of *Ochlerotatus japonicus*. *Journal of the American Mosquito Control Association* 19(4):376-381.

Sudia, WD, and RW Chamberlain. 1967. *Collection and Processing of Medically Important Arthropods for Arbovirus Isolation*. Centers for Disease Control, PHS, USDHEW, Atlanta, GA. 29 pp.

SCVC. 2001. *2002 Annual Plan of Work*. Suffolk County Department of Public Works, Division of Vector Control.

USGS. 2004. *West Nile Virus Project*. National Wildlife Health Center. http://www.nwhc.usgs.gov/research/west_nile/west_nile.html

Walker, ED, and WJ Crans. 1986. A simple method for sampling *Coquillettidia perturbans* larvae. *Journal of the American Mosquito Control Association* 2:239-240