APPENDIX E

Nuisance Control and Public Health Protection

Considerations Regarding Mosquito Control for Public Health Maintenance and Nuisance Abatement

Prepared by Cashin Associates, PC

Organized mosquito control was initiated around 1900 for the explicit purpose of controlling mosquito-borne diseases, especially malaria and yellow fever. The hey-day of mosquito control effort, in terms of manpower commitment, was in the early 1930s during the massive mosquito ditching projects, when malaria had mostly been eradicated from the country – and the targets of control were largely the salt-marsh and other flood water mosquitoes, not *Anopheles spp*. These efforts were thus conducted to improve the quality of life in areas where pestiferous mosquito biting occurred. The difference in ends of the two efforts is usually described as one being for human health protection, and the other for nuisance control.

It is far from clear that these efforts can be clearly distinguished. This is true in terms of historical perspectives, and also in how modern mosquito control efforts are justified.

Mosquito-borne disease is one of the most serious health threats in the world. There may be up to a half-billion cases of malaria each year, resulting in several million human deaths. This particular disease requires a human host. The mosquito serves as the vector for the disease, and transmits the contagion from person to person with extraordinary efficiency. A single human host can result in hundreds of new infections, far beyond the impact of ordinary air-borne human illnesses. Two other mosquito-borne diseases for which humans are hosts, yellow fever and dengue fever, although not spread as easily, have high fatality rates and cause immense suffering in those that die and also in those that recover.

In the US, the primary public health concern with mosquitoes in the 21st Century is encephalitises spread to humans from other hosts by the mosquitoes. This process makes the spread of disease less swift than when humans are hosts. Where these arboviruses are not endemic, outbreaks tend to be affected by environmental factors that control either primary host or mosquito vector numbers. Some of these diseases have very high fatality rates (exceeding 50 percent). West Nile virus, the latest of the diseases to appear here (1999), seems to be atypical, however, and may become endemic for the country as a whole.

Mosquitoes are especially unwanted pests. This may be due to the legacy of diseases; however, it is also likely due to the combination of whining approach, often-times large numbers, fairly even distribution so that avoidance can be difficult, and the pain associated with bites. Many areas of the country are sometimes described as being uninhabitable prior to mosquito control efforts, the manufacture of effective screens, and the widespread use of air conditioning – although it is more accurate to suggest that development would not have occurred as widely and to the current degree, rather than suggesting no one would live in these areas. Florida is especially called out in this regard. The South Shore of Long Island was similarly described by some.

Some other insects can cause human irritation is similar fashions. Black flies can be overwhelming in terms of biting rates in some melt-water areas, but infestations rarely last longer than a month. Horse flies, green head flies, and other large flies can have painful bites, but they tend to fewer in number, and easier for humans to avoid. Wasps and bees can have exceedingly painful stings, but are even easier to avoid than flies (in general). Midges and gnats can be extremely prevalent, but tend not to have painful bites or spread disease.

Ticks may cause human problems to the same degree that mosquitoes do. Ticks also spread human disease, inflict painful bites, and, in some areas of the country, may be difficult to avoid, even on one's own property. If ticks carrying Lyme disease become as common in backyards as mosquitoes are, ticks may also be subjected to government control efforts. However, the perception of tick-borne disease has not risen to that level of concern generally, either nationwide or even with Suffolk County (an epicenter of Lyme disease prevalence).

Most mosquito control professionals view control efforts as being conducted both for protection of human health and as nuisance control. Instead of viewing these as two distinct efforts, most of these professionals treat the approaches as endpoints along a continuous line. The line describes the focus of a particular control effort. Sometimes it is intended mostly as a control of nuisance, and other times it is a matter where disease control is of paramount importance.

Most mosquito control agencies use pesticides as part of their control efforts. Many people are not comfortable with the use of chemicals to control mosquito populations. Concerns are sometimes raised regarding studies that show potentials for impacts to human health or the environment. Often, however, the concerns seem rooted not so much in knowledge of specific science that identifies impacts, but rather in general worries over the use of synthetic compounds. There is a philosophic stance that assumes that because synthetic chemicals are not natural, they must have deleterious effects on natural systems. Synthetic pesticides usually provoke a stronger reaction, because not only are the compounds unnatural, but their intent is to damage some form of life.

There is truth to the notion that many synthetic chemicals can damage natural systems. It is also true that broad-spectrum, long-lived pesticides have had unintended effects on many organisms and ecosystems. Some pesticides have acute, toxic effects on humans. Most modern pesticides do not, although there is some evidence of increased rates of emergency room treatments of symptoms that could signal pesticide effects, following widely-publicized episodes of pesticide applications. However, less publicized events do not seem to generate the same kind of human reaction, suggesting that some of the impacts may be due to psychosomatic reactions to potential pesticide exposures. This does not mean that the reported illnesses did not occur, but that the cause of the maladies may be more complex than exposure to a certain concentration of a particular chemical.

Pesticides currently used for mosquito control undergo toxicity and environmental impact testing prior to receiving permits for use. Government regulators have reached an expressed judgement, by setting allowable usage rates and conditions, that these chemicals do not have unacceptable impacts to human health and the environment.

However, regulators also specify that applicators need to make further decisions regarding the risks that use of the chemicals may have some defined impact, either to people or the environment. These further assessments of risk tend not to be quantitative. Rather, they are often derived from underlying, general tenets of public policy. Thus, the Ministry of Health of British Columbia has determined that it will not apply adulticides to kill mosquitoes until there are human cases of West Nile virus, while other jurisdictions prophylacticly use adulticides to prevent human cases from occurring. In one instance, government officials believe the public (as a whole) will not accept the risks associated with pesticide use until the disease is expressed in people; in the other case, officials believe that the public (as a whole) will not accept incidences of a disease that might have been prevented if pesticides had been used.

If this latter attitude is extended to its furthest reach, then all mosquito control efforts can be justified in terms of disease control. This is because mosquito-borne human disease is, of course, spread by mosquitoes that bite people. Laboratory tests or field collections generally have shown that nearly every human biting mosquito has some ability to carry disease of one kind or another, and so potentially can infect humans. Different genuses and species within genuses have different capabilities, so that *Anopheles* is the only genus that carries malaria, and *Culex spp*. seem to be the best at transmitting West Nile virus. Not all mosquitoes are as capable of infecting people, or at harboring particular viruses, certainly. However, some potential for disease transmission is associated with nearly every tested human biting mosquito species. In addition, because most diseases must infect adult mosquitoes prior to transmission to humans, and that infection almost always occurs through a blood meal, it is older mosquitoes that have already fed (parous mosquitoes) that need the most control. Therefore, early intervention holds the promise of reducing, at all times, the number of older mosquitoes, and so should reduce overall chances of human disease incidence. Thus, larval control and even adulticiding of general mosquito populations, even prior to the detection of disease in monitored mosquitoes, can be rationalized as disease prevention activities. In some important instances, the benefit is almost infinitesimal, because, for some species, adult mosquitoes can have mortalities rates approaching 50 percent per day (which means there are almost no mosquitoes that are two weeks old, and so early control may not reduce parous adult presence when disease is actively circulating).

But mosquitoes are also generally acknowledged to be capable of reducing quality of life. This is often called nuisance. The term “nuisance” is often equated with annoyance, which does not seem to be a very important problem. However, nuisance also can be understood to be “unbearable.”

Unbearable is not a quantitative description. Various approaches to what are acceptable biting impacts from mosquitoes, and what are not acceptable, have been assayed. Some jurisdictions have established numerical triggers. These triggers were often based on landing rates (the number of mosquitoes biting – or attempting to bite – a human over a predefined length of time), although the determination of landing rates is now often considered to be unacceptable for health and safety reasons. Proxies for landing rates, such as mosquito trap data, may be used instead.

Another approach is to address mosquito problems contextually. For example, areas where people camp, hunt, or fish, may not have mosquito nuisance problems, because people in these settings are seeking "natural" experiences – and mosquitoes are a part of nature. The concept of unaltered nature is part of the underlying rationale for the general lack of mosquito control by the National Park Service, and also seems to be part of the reasoning behind the policy change for National Wildlife Refuges, which are now seeking to eliminate pesticide usage for mosquito control. People living in rural settings also have different expectations regarding insect presence and acceptable levels of discomfort, compared to other residential settings. In many suburban environments, however, where houses were purchased with the expectation that the surrounding property would be available for recreational use, an inability to use outdoor areas is perceived as a loss of inherent property rights. The value of these properties would certainly be less if, for example, there were months-long time periods when outside activities could not occur due to mosquitoes. The same perception occurs for those going to resort areas, where the attraction of the particular resort is based on outside access and activities.

Thus, the issue of nuisance can become a quality of life issue, where there is little tolerance for mosquitoes. Besides the unquantifiable issues of enjoyment and use of property, there will also tend to be economic impacts, so that property values may be diminished, or resort rental rates decreased. Similarly, hotel occupancy rates or other measures or tourist attraction may fall. Because government appears to have tools to eliminate this problem, expectations can arise that government should try to address it. Actions may be taken by government of its own volition, or constituent pressures may result in elected officials taking steps to implement programs.

Each incidence of mosquito control needs to be understood as having an element both of control of nuisance and also of control of human health concerns. Treatments prior to clear demonstration of virus presence are clearly weighted towards nuisance abatement, yet they also may reduce disease transmission risks. Treatments in response to virus detection or active human illness clearly focus on reduction of the disease threat, but will also abate the lifestyle impairments that may be associated with the targeted mosquito population. It will be exceeding rare that control efforts can clearly be defined as being purely for health threat reduction or solely to control a nuisance.

Efficacy of the steps taken to reduce mosquitoes must be understood. If the control efforts do not have any impact on mosquito numbers, or, in the case of public health preservation, do not lead to reductions in heath risks, then it could be argued that the control effort was unnecessary. Well-run mosquito control programs can demonstrate reductions in mosquito numbers, and generally show reduced arbovirus presence after treatment (if the virus had been detected in mosquito populations). However, demonstration that absolute disease risk had been reduced or eliminated, or that nuisance had been adequately abated, generally depends on indirect calculations (for health risk reductions) or qualitative measures (for nuisance, unless nuisance definitions are based on absolute quantitative triggers). Thus, many who have concerns regarding the impact of mosquito control efforts have those concerns reinforced when subjective assessments of efficacy are offered as rationales for continuing to treat mosquitoes.

The Long-Term Plan is aware of these issues, and will attempt to address many of them. For example, a quantitative assessment of the impacts to human health and the environment from the use of pesticides for mosquito control is part of the project. The risks from control measures will be compared to those calculated for the risk of mosquito-borne disease. That assessment should make it clear whether the benefits associated with the prevention of disease outweigh the risks to human health from pesticides – and, if that is the case, the degree to which it occurs. If the risks from pesticides are approximately commensurate with the benefits of disease control, then sparing use of chemicals would be indicated, and only when there is clear evidence that people's lives are at risk. If, on the other hand, the human health impacts from pesticides appear to be vanishingly small compared to the risk of disease, then control of disease risk is clearly indicated whenever transmission is plausible.

Ecological impacts, if demonstrable with pesticide use, cannot be clearly compared to human health risks from disease. This comparison will need to be understood as a tradeoff between benefits to people but losses of ecological values. If the scope of the impact to the environment is smaller, then any decision to make the choice to control mosquitoes, despite environmental impacts, is easier. Nonetheless, this decision must be made on the basis of some underlying public policy, not simply on the basis of compared risks, as the comparison cannot be made in terms of similar valuations.

Similarly, any decision to allow for predominantly nuisance control abatement, in comparison to potential health impacts, is also made through some underlying public policy choice. It is easier to accept nuisance control if the pesticides impact is very small – or to reject nuisance control if the impact from pesticides on human health is found to be relatively large. However, a decision will need to be made based on the perception of what the population of the County, as a whole, is willing to accept and support.

Nuisance control in terms of environmental impacts, can, in a sense, be made quantitatively – although both proponents and opponents may reject the basis of comparison. Environmental impacts can potentially be evaluated in terms of economic impacts (although it is difficult to do to the satisfaction of all interested parties); similarly, it may be possible to discuss nuisance impacts in terms of losses in housing values, tourism impacts, and similar measures. As mentioned, however, proponents of nuisance control can express forceful arguments that the economic discussion does not capture the full scope of losses associated with mosquito infestations, just as those concerned with impacts to the environment will state that economic evaluations almost never give full due to the impact of ecological change.

Nevertheless, by fostering a more complete and more open discussion of these issues, it is hoped that more agreement can be reached regarding acceptable conditions for mosquito control in Suffolk County. These conditions must clearly involve notions of nuisance abatement and disease risk management. The Management Plan will detail the consultant team's perception of an optimal approach that minimizes human and environmental impacts from control measures, and maximizes the public health and quality of life benefits within that understanding of risk. It is also possible, however, that this technically-oriented approach may not clearly understand the how the public will evaluate the same data, and that necessary adjustments to the plan will be implemented following public comment and criticism.

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

Task Four
Nuisance Versus Vector Control Decisions: Public Health Implications for Suffolk County

Prepared for:

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Suffolk County Department of Health Services
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SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS MANAGEMENT LONG - TERM PLAN AND ENVIRONMENTAL IMPACT STATEMENT

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TABLE OF CONTENTS

Tables

Appendix

Executive Summary

No clear lines can be drawn to distinguish nuisance from vector control. Nonetheless, time of year and physiological age of known vectors can be used as criteria for classifying responsible mosquito control decisions. Surveillance programs to identify the insects to species, map their larval distribution, measure their long-term population curves, and monitor their physiological age place important biological information in the hands of mosquito control professionals. Health related information on presence or absence of virus in both mosquitoes and birds provides an added matrix of data to measure public health risk as the season advances. New Jersey has found that Eastern Equine Encephalitis (EEE) risks can be substantially reduced by using carefully targeted adulticide applications, when warranted, to supplement vigorous water management and larval control efforts. It is true that EEE transmission factors are relatively simple compared to those involved in West Nile Virus (WNV). For Suffolk County, for example, essentially all mosquitoes that cause nuisance conditions are potential vectors of WNV. This means the numerical trigger developed for EEE in coastal New Jersey is not applicable for WNV control decisions. However, the general principles for identifying conditions where control will produce primarily human health protection, as opposed to mostly providing relief from biting nuisance. However, even in the absence of simple trigger values. Mosquito control is a professional endeavor that uses science in its decision making processes, which separates it widely from nearly all other methods of pest control.

1. Introduction

Mosquitoes relate to human health and well being in two very different ways which can directly affect the approach used to control biting populations. Their host seeking behavior makes them direct agents of discomfort with no parasitic agents involved. This is often referred to as nuisance or annoyance that interferes with daily activities. When mosquito annoyance is especially high, people are driven indoors and cannot take part in a number of summer recreational activities. Mosquitoes also have the ability to serve as vectors of organisms that cause clinical disease. Malaria, yellow fever, and some encephalitis viruses are examples of mosquito-borne diseases that can kill. Mosquito-borne filariasis and a number of viral agents are non-lethal but produce a diagnosable illness that is caused by a parasitic agent. Multiple mosquito bites, in the absence of a parasitic agent, often go beyond nuisance, causing lasting welts, diagnosable dermatosis, and allergic reactions, particularly in children. Mosquito control agencies often have an unclear mandate in terms of nuisance versus vector control.

Mosquitoes serve as biological vectors of the mosquito-borne diseases they transmit. The parasites that use this group of insects either propagate (propagative transmission), change in form (cyclo-developmental transmission), or do both (cyclo-propagative transmission) within their insect host (Harwood and James, 1979). To complete any one of the life cycle types they undergo within the insect, the parasites require time. As a result, an incubation period ranging from several days to several weeks is required before a parasite ingested by a mosquito is ready to be transferred back to a vertebrate host. "Old" mosquitoes, therefore, are considerably more dangerous than "young" mosquitoes as vectors of disease, and the age of the target population becomes an important criterion when assessing nuisance versus vector control approaches for intervention.

Simplistically, control directed toward mosquitoes that do not contain parasitic agents could be designated as "nuisance control." Alternately, control directed toward populations known to be carrying parasitic agents would be correctly designated as "vector control." Unfortunately, disease surveillance cannot always distinguish whether the mosquitoes constitute a nuisance or a vector at a specific point in time. Theoretically, the older a mosquito is, however, the greater the probability that it has made contact with a parasitic agent and is capable of passing that parasite on. Techniques have been developed to determine exactly how many blood meals an individual mosquito has obtained. Techniques are also available to age-grade wild mosquito populations and rank them in terms of their immediate vector potential. This report examines blood feeding in mosquitoes in terms of changes that take place internally from multiple blood meals, and methods public health personnel have designed to use that information to identify vector populations. The information is presented as a guide for nuisance versus vector control decisions and the protection of public health.

2. An Overview of Blood Feeding in Mosquitoes

Mosquitoes belong to a group of haematophagus Diptera that exhibit synchronization between blood feeding and ovarian development known as "gonotrophic concordance." The term was first proposed by Swellengrebel (1929) working with the *Anopheles maculipennis* complex of malaria vectors in Europe and represents one of the most unique life style strategies in the animal kingdom. Insects that exhibit gonotrophic concordance feed on both plant juices and blood but do not, technically, use blood as food. Carbohydrates from plants are used for all of their energy requirements. Thus, mosquitoes rely on nectar and other sugars to fuel daily activities, including flight. Protein from blood, however, is a prerequisite for egg production in these insects, and at least one blood meal is required for each batch of eggs that is laid. Since male mosquitoes do not lay eggs and have no need for a protein source, only female mosquitoes bite. A female mosquito could survive an entire life time without taking a blood meal, but the life style strategy she abides by would not allow that insect to reproduce.

The ovaries in insects that undergo gonotrophic concordance enter a resting stage between blood meals where nurse cells provide nutrition for the undeveloped oocytes (Nicholson, 1921; Feinsod and Spielman, 1982). Acquisition of blood releases a gonadotrophic hormone that triggers the ovarian developmental process. Protein from the blood meal builds yolk which allows the eggs to grow rapidly within the insect. The more than 100 eggs within each ovary pass through progressive stages of development originally described by Christophers (1911), but re-described by numerous authors (Sokolova, 1994), changing from round in shape to distinctly elongate. The entire process from blood meal acquisition to oviposition takes only several days at favorable ambient temperatures, returning the mosquito to a host-seeking mode to start the cycle over.

The gonotrophic cycle that takes place in mosquitoes includes three biological phases which regulate the daily activities of individual insects:

1) the search for a host and acquisition of the blood meal,

2) digestion of the blood, oocyte release from the resting stage, and development of one full batch of eggs, and,

3) a search for suitable larval habitat and oviposition of the fully developed eggs (Detinova 1962).

When the first gonotrophic cycle is completed, a second batch of oocytes enters a resting stage and relies on a similar biological sequence to reach full development. Host seeking, blood meal engorgement, egg maturation, and oviposition continue repeatedly until death terminates the repetitive reproductive cycle.

3. The Concepts of Physiological Age and Vector Potential

Age in living organisms can be measured in two very different ways. Calendar age represents the number of days or years the animal has survived and is the most common way to assess age in warm blooded animals. Physiological age is assessed from the accumulation of irreversible changes in the organism as a consequence of normal life processes (Beklemishev, 1962) and is more meaningful for cold blooded animals where temperature regulates ongoing life processes. As an example, the instar of a larval mosquito represents the physiological age of that organism irregardless of the number of days or weeks it took that larva to attain each molt. A 4^{th} instar larva of a summer floodwater mosquito species is approximately four days old, while a 4^{th} instar larva of a species that overwintered as an immature might be six months or more old. Physiologically, however, the four day-old floodwater larva is the same age as the six month larva. Both represent insects that are ready to enter the pupal stage of development, the next step in the process of becoming an adult.

The number of gonotrophic cycles completed by the organism is used to classify the physiological age of an adult mosquito, representing significant biological events that are internally regulated by temperature. Each batch of eggs that are laid by a female mosquito leaves a number of irreversible changes within the ovaries that can be catalogued and measured to assess physiological age. Russian scientists pioneered studies in the 1950s to physiologically age-grade mosquito populations for vector potential purposes, for studies designed to minimize malaria transmission.

Newly emerged mosquitoes possess ovaries with follicles that have never been broken from their resting stage. Mosquitoes that have completed a gonotrophic cycle have ovaries that have expanded enormously and then shrunk back down. The process of ovarian development, oviposition, and return to the resting stage leaves a number of telltale signs in reproductive tract (Detinova, 1962; Clements and Boocock, 1984). Relatively simple observations of the ovaries reveal that the reproductive tract has passed through at least one gonotrophic cycle. Techniques are also available to determine the exact number of gonotrophic cycles an individual female mosquito has undergone (Polovadova, 1941; Rosay, 1962; Giglioli, 1965).

4. Parity and Nulliparity as Basic Classifications of Physiological Age

4.1 Introduction

Mosquitoes that have passed through at least one gonotrophic cyle are termed "parous", meaning that they have laid at least one batch of eggs. Since egg production requires blood, parous mosquitoes have imbibed blood from at least one animal host in nature. Mosquitoes that are newly emerged have never laid eggs and have never fed on blood. The term "nulliparous" is used to describe a mosquito that is searching for its very first blood meal host. Nulliparous mosquitoes cause annoyance when they come to bite, but do not pose an immediate health threat. Having never fed on blood before, the mosquitoes cannot serve as carriers of any of the mosquito-borne disease parasites known to occur in the northeast. Parous mosquitoes, however, have obtained at least one previous blood meal, which increases vector potential substantially. More importantly, the time it took the mosquito to complete the gonotrophic cycle(s) provides a period for biological incubation of viruses, protozoa, or nematodes that might have been taken in by the mosquito along with the blood meal. Multi-parous specimens pose the greatest health risk because they have made contact with multiple hosts and have survived long enough for parasitic agents to undergo complete development.

4.2 Age-grading Mosquito Populations to Measure Vector Potential for Mosquito Control Purposes

Age-grading of mosquito populations was developed through malaria research to keep *Anopheles* vectors in tropical areas physiologically "young." This minimizes malaria transmission by keeping the number of potential vectors below density levels required to maintain the disease. The rationale allows for the presence of *Anopheles* mosquitoes in areas where malaria is known to be endemic without a significant influx of new malaria cases. Malaria transmission requires relatively high densities of multi-parous vectors because the plasmodial parasites require a minimum of nine days within a mosquito before they can be transmitted back to a human host (Burkot and Graves, 2000). As a result, control strategies, using residual insecticides, were developed to combat the disease by specifically targeting older mosquitoes to keep biting populations primarily nulliparous or uni-parous (Harwood and James, 1979).

Malaria transmission in tropical areas is ongoing because vector populations breed continuously during most of the year. The arthropod-borne diseases that occur in the continental United States show distinct seasonal patterns, particularly those that pose problems in the northeast. In this country, the term vector control is used to describe control efforts enacted during public health emergencies that target specific vector species for the prevention of disease (Reeves and Milby, 1990; Rice and Pratt, 1992). Many of the age-grading strategies developed for malaria research in tropical areas have direct application for vector control purposes in temperate zones and some have been specifically developed for use in the northeast (Hitchcock, 1968; Crans, 1976; Crans and McCuiston, 1993).

4.3 Age-grading Floodwater Mosquito Populations for Vector Control Purposes

The life cycle strategy demonstrated by floodwater mosquitoes in temperate areas is well suited to age-grading of biting populations for the purpose of vector control during periods of public health emergency. High mosquito populations are usually an indication of a fresh emergence in brooded species like *Aedes vexans*, a fresh floodwater species, and *Ochlerotatus sollicitans*, a floodwater mosquito that emerges synchronously in large numbers from saltmarsh habitats (Crans, 2004). The majority of individuals in the biting population are seeking their first blood meal immediately after an emergence, and cause considerable annoyance when large numbers of adults are on the wing. The potential for disease transmission is minimal at this point in time, however, because the nulliparous mosquitoes that are causing the problem have never fed on blood before and have not made contact with any of the parasitic agents that produce human disease. Control of nulliparous biting populations is often instituted to minimize nuisance and is usually driven by citizen complaints. As the population ages, the number of host-seeking mosquitoes declines to levels where complaints drop markedly, because the brood is dying off from natural causes. Because of their lesser numbers, older biting populations are often ignored even though they contain the mosquitoes most likely to be carrying the agents of human disease. The individual mosquitoes which make up the biting population two to three weeks after an emergence are all parous and are currently seeking their second or third blood meal. Table 1 compares factors that promote increases in vector potential over time. Control efforts specifically directed toward parous populations of biting mosquitoes is vector control by definition. Vector control against targeted populations is most effective when surveillance has detected virus activity in the treatment area.

Table 1. Epidemiological relationships during periods of high and low floodwater mosquito biting populations.

High Biting Populations	Low Biting Populations
Recent Emergence has taken place	Brood is dying off
Majority of population is nulliparous	Majority of population is parous
Mosquitoes seeking 1st blood meal	Mosquitoes seeking second or third blood meal
Annoyance is considerable	Annoyance can be minimal
Potential for contact with pathogens is minimal at this point in time	Potential for contact with pathogens much greater because of prior blood meal(s)
Vector potential is low	Vector potential has risen
Control usually required to abate nuisance	Control may be required for prevention of disease

If broods of floodwater mosquitoes did not overlap, vector control decisions could be made on a sliding time scale with health concerns escalating as the brood ages. Floodwater broods, however, frequently overlap, producing biting populations that contain nulliparous, uni-parous, and multi-parous specimens. Crans (1976) proposed the use of parous landing rates as a surveillance technique to age-grade and monitor *Oc. sollicitans* populations for vector control. Crans and McCuiston (1993) report the results of a cooperative encephalitis prevention program where age-grading techniques have been integrated into New Jersey's overall vector control efforts.

4.4 Using Parous Landing Rates for Vector Control

Most floodwater mosquito species are opportunistic feeders that bite avidly during daylight hours when hosts enter their resting sites. Landing rates are often used to measure the size of host-seeking populations and their distribution within potential treatment areas. The technique records the number of mosquitoes that attempt to bite over a standard unit of time. Details on how mosquito control agencies collect landing rate data can be found in Appendix 1. Landing rates per minute are usually used for aggressive salt marsh mosquitoes because biting densities are normally high. Longer units of time can be used for some of the less avid blood feeders or when mosquito density is low. Landing rates of 10 or 20 per minute are common for salt marsh mosquitoes sampled within one mile of a coastal breeding site (Crans et al., 1976). Immediately after a brood has emerged, counts 10 times that high can often be obtained close to the emergence site.

The technique of "Parous Landing Rates" incorporates age-grading to landing rate collections for added information that might lead to vector control. No fewer than 20 host-seeking mosquitoes are collected after multiple landing rate counts have been taken. The mosquitoes are frozen at the collection site and later dissected in the laboratory to determine the percentage of the biting population that is parous. Details on dissection techniques used for age-grading mosquito populations can be found in Appendix 1. The number of parous mosquitoes coming to bite per unit time is calculated by multiplying the landing rate by the parous rate.

5. Seasonal Considerations Regarding Nuisance versus Vector Control

The viral agents that produce Suffolk County's mosquito-borne disease problems show distinct seasonal patterns that are regulated by biological events. Cold weather terminates active transmission by mid-October in most years and the viruses enter quiescence in either the mosquito vectors or the vertebrate hosts. Mosquito activity the following season is minimal until night-time temperatures rise above 60^{o} F during May or early June. EEE and WNV begin in a benign cycle that is limited to the avian reservoirs of the viruses and a number of fixed bird feeding mosquito species. Virus levels are rarely amplified to where mammalian bridge vectors can pick them up until late July or early August. As a result, mosquito control during spring and early summer targets mosquito populations that impact outdoor activities. As the summer progresses and virus levels become amplified, however, mosquito control concerns shift to focus on public health protection. It is difficult to predict the exact point in time where vector control is required to abort the transmission cycle. Well designed virus surveillance programs provide early indications for potential health problems by testing mosquitoes, birds, and sentinel animals for evidence of virus amplification and transfer to bridge vectors before human and equine cases have occurred. Table 2 provides examples of age grading information that can be used to make responsible vector control decisions as the season advances. Both early and late season assessments have been included to separate nuisance from vector control.

6. Other Considerations Regarding Nuisance Versus Vector Control

Vector control, by definition, is directed against mosquito species that have been implicated on epidemiological grounds as natural carriers of disease to reduce the risk of pathogen transfer to humans and domestic animals. Until WNV appeared in 1999, the number of confirmed vectors of human pathogens in the northeast were few, making targeted nuisance versus vector control decisions relatively simple. *Ochlerotatus sollicitans, Aedes vexans, Oc. canadensis and Coquillettidia perturbans* are listed as the primary bridge vectors of EEE to humans in the northeast (Callisher, 1994). Most of the other mosquitoes on Suffolk County's list of mosquitoes of concern were considered biting pests rather than vectors, including the *Culex* complex of mosquitoes (*Cx. pipiens, Cx, restuans* and *Cx. salinarius*). WNV, however, has since been isolated from 43 different mosquito species, including virtually all of Suffolk County's human biting pests (CDC, 2005). The arrival of WNV and its occurrence in an ever widening range of domestic pest species, including the *Culex* complex, broadens the scope of vector control once surveillance programs have identified virus activity in the county.

One issue that has not been fully determined is the impact that large broods of uncontrolled salt marsh mosquitoes might have on population size later in the season. It is plausible that uncontrolled egg deposition early in the season produces larger broods later in the summer, but no data are available to support that claim. Most mosquito control superintendents believe that large broods in May and June point toward high populations in the fall, and so nuisance control (guided by proper surveillance) promotes efficient vector control when it is needed for public health protection. All agree that season long larval control of salt marsh mosquitoes is absolutely necessary. Adulticiding reaches a very small percentage of the overall mosquito population because applications are limited to areas where biting mosquitoes impact humans. The vast majority of adult salt marsh mosquitoes survive to lay eggs, even when adulticiding is conducted regularly. Larval control is conducted over a much broader area of available habitat and is essential to minimize the number of late season adults that can emerge after EEE and WNV have been amplified in local bird populations.

Other public health risks can develop if populations of any mammalian biting species are allowed to age without intervention. Scores of mosquito-borne animal parasites occur in wildlife, including babesia, leucocytozoans, spirochaetes, viruses, and filarial worms (Beaver and Orihel, 1965). Although these parasitic agents are considered benign when accidentally introduced to humans, it is poor public health policy to encourage transmission of living organisms to humans. More importantly, allowing large broods of mosquitoes to emerge to feed on humans forces the public to rely on repellents for relief. Most adults overapply commercially available repellents, and consequences regarding health risks to children have not yet been fully researched.

Table 2. Examples of age grading information useful for Vector Control decisions.

Landing Rate (Total Mosquitoes Landing/Min.)	**Parous Rate** (From Ovarian Dissections)	**Parous Landing Rate** (Parous Mosquitoes Landing/Min)	**Early Season Vector Control Assessment**	**Late Season Vector Control Assessment**
40	0%	0	New brood has emerged. Annoyance is severe but vector potential is minimal. Nuisance control would based on citizen complaints.	New brood has emerged. Surveillance data have identified virus activity in amplification vectors. Vector control is needed for public health reasons.
30	10%	3	Possibility 1 - New brood has been added to an old residual population. Older portion of the population should die off naturally. Vector potential is minimal. Nuisance control would be based on citizen complaints.	Vector control required for reasons listed above
30	10%	3	Possibility 2 - New brood has emerged with 10 percent now seeking 2nd blood meal. Vector potential is minimal. Nuisance control would be based on citizen complaints.	Vector control required for reasons listed above
20	50%	10	Landing rates high with rising parous rate. Landing rates should be monitored closely over next several days. Control may be required to eliminate bites	Vector control essential to prevent further aging.

			from old mosquitoes	
10	90%	9	Majority of biting population has fed on blood before. Control would be justified on the basis of population age.	Classic case for Vector Control for public health purposes underscored by surveillance data showing late season virus activity.
2	100%	2	Brood is in final stages of dying off. No control required.	Brood is in the final stages of dying off. Spot treatments may be required to eliminate residual biting populations.

7. New Jersey's Experiences with a Vector Control Approach

In 1975, the New Jersey Agricultural Experiment Station (NJAES) developed the New Jersey Vector Surveillance Program, a monitoring effort directed toward EEE and its mosquito vectors in coastal areas of the state (Crans et al., 1976). The program was funded by the New Jersey State Mosquito Control Commission and has grown into a cooperative effort that includes the state departments of Health, Agriculture, Environmental Science and the county mosquito control agencies that benefit from the service. The objectives of the program are two-fold:

1) to determine if EEE virus is present in nature at a variety of established study sites where the disease has been detected in the past, and,

2) to determine the risk for transmission to humans as the season progresses and make that information available for vector control decisions.

The mosquito, *Culiseta melanura*, is used as the main indicator of EEE virus in nature. This bird feeding mosquito is collected weekly from six permanent study sites and tested for:

1) population levels as the season progresses,

2) deviations in population levels from the long-term mean, and,

3) levels of EEE in the bird populations it is feeding on.

All information is entered into a database for collation. The information, including graphical interpretations, is made available weekly to mosquito control agencies in the state.

Equine cases are the indicator for accelerated control of *Coquillettidia perturbans*, the primary vector of EEE at inland areas of the state (Crans et al., 1986). This information is provided by the New Jersey Department of Agriculture, which requires all state veterinarians to report suspect cases of EEE in horses as soon as possible. This allows mosquito control agencies to target their vector control activities to areas where known vectors of the disease occur in greatest numbers.

In coastal areas of the state, the physiological age of *Oc. sollicitans* is used as the main indicator of risk for transmission to humans. Field technicians from NJAES monitor *Oc. sollicitans* populations at numerous stations along the New Jersey coast from June through October, and take multiple one minute landing rates to assess population size. They also collect and freeze specimens for physiological age determinations. The number of parous mosquitoes coming to bite is obtained by multiplying the landing rate collected at the field site by the parous rate determined by ovarian dissections. Mosquito control agencies are advised when parous landing rates exceed five per minute in areas of the state where EEE is known to be circulating in *Cs. melanura*. The agencies then deploy staff to spot perform additional ovarian dissections and to check for residual biting populations. If the data support them, decisions to perform vector control follow, as necessary, for public protection.

Crans and McCuiston (1993) reported that 49 human cases of EEE were confirmed in the 15 years prior to the program's inception. Only three have occurred in the 15 years that followed. Vector control decisions were based on the examples given in Table 2, using both age and known presence of virus as the primary criteria to reduce vector potential of biting populations of salt marsh mosquitoes. Scarcity of funds in the middle 1990s forced New Jersey's coastal counties to assume most of the duties using physiological age dissections to determine parity in late season *Oc. sollicitans* populations. A total of only three additional human cases in the twelve years that followed point toward success of the vector control approach to reduce disease transmission to humans.

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Appendix

Techniques used to Assess Physiological Age in Mosquito Populations

Landing Rate Counts

Rationale: A number of aggressive human biting mosquito species will opportunistically take a blood meal whenever suitable hosts invade their resting habitat. The behavioral trait can be used to obtain an immediate population index at multiple locations during daylight hours to supplement the regional light trap data obtained from ongoing surveillance programs. Landing rates are most often used for *Oc. sollicitans* in areas close to their coastal habitat. The technique is also applicable for *Ae. vexans* and *Oc. trivittatus*, two opportunistic fresh floodwater species that readily leave resting sites to bite during the day.

Habitat: Knowledge of suitable resting habitat for the species being monitored is essential to accurately use landing rates to determine population size. Standard collection sites set in ideal resting habitat are recommended for comparative purposes but random sites can be sampled to measure nuisance levels in response to complaints. Landing rates for *Oc. sollicitans* are generally conducted within ½ mi. of their salt marsh breeding habitat, the zone where the numbers are generally highest along the inland transect where they host seek. Pockets of short grass in open fields hold the greatest numbers. If wind is a factor, similar habitat along the ecotonal edge of woodland barriers provides ideal resting sites where landing rate counts can be taken. The first 25 yards of a path that leads from field into forest habitat can be productive, particularly when wind keeps the biting population low. Moist areas within dry fields hold the largest resting populations because humidity is required for day time survival. Areas along the ecotonal edge that support mosses provide the best resting habitat and are usually specified as permanent landing rate sites by mosquito control agencies.

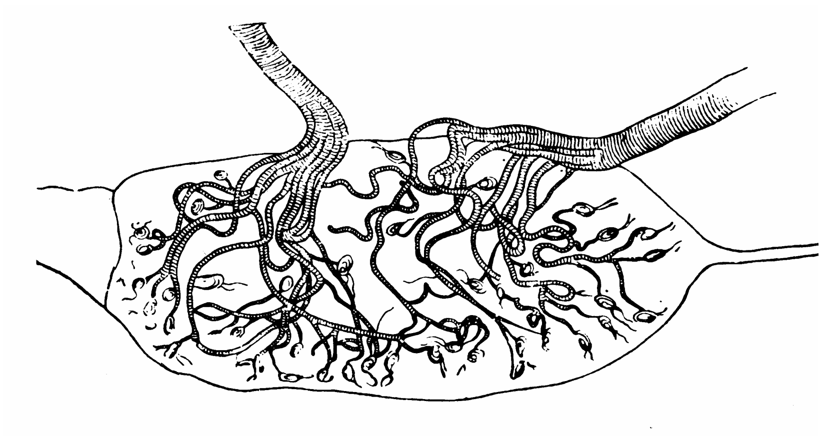
Procedure: Landing rates can be taken by a single individual, although some agencies prefer to use teams. Repellent can be used on the upper body to minimize bites on open skin, but none should be applied below the waist. Light colored clothing makes counting somewhat easier but white reflects too much light and should be avoided. Day time biters use their eyes to locate an opportunistic host so motion is important by the sampler. The technician should walk through the vicinity where the count will be taken to stir up the mosquitoes but avoid the exact spot chosen for the count until the very end. Host seeking day time biters will follow a moving host so it is best for the sampler to turn 180^{o} just before the count is made. Mosquitoes that land below the waist in view are counted over a time span of one minute. Timers that give an audible signal prevent the counter from having to check a watch during the collection period. Mosquitoes disturbed from resting sites generally land low on the body and move upward in a steady wave. Counts are made by repeatedly scanning the lower torso looking as far to the back of the legs as is practical without unnecessary movements. Unless populations are very low, exact counts are hard to achieve because some specimens will be missed and others will be counted twice. Technicians experienced with landing rate methodology quickly learn to make meaningful estimates; however, estimates should be always be confirmed by actual counts. The procedure should be repeated three times at each site that is sampled with the mean recorded as the rate for that area.

<u>Confirmation of Species</u>: Once the count is made, technicians should move about and collect a minimum of 10 specimens from the general vicinity for identification to species. This allows the laboratory to break down collections that contain more than one species and calculate meaningful rates based on percentage.

<u>Parous Landing Rates</u>: If physiological age of the host seeking population is of interest, technicians should collect a minimum of 20 specimens which should be frozen on dry ice at the field site and returned to the laboratory for parity analysis. Parous landing rates are obtained by multiplying the original landing rate count by the percentage that are parous (i.e. 10 mosquitoes landing per min. with a 10% parous rate = 1 parous mosquito landing per minute of time).

Parity Dissections

Rationale: Host seeking mosquitoes always have ovaries that are reduced in size with ovarioles in a resting stage that require protein for further development. Parous mosquitoes have laid eggs at least once and have ovaries that have expanded and then shrunk back down. Nulliparous mosquitoes have never laid eggs and possess ovaries that have never expanded. The tracheal system that supplies oxygen to the cells of the ovaries can be used as an indicator of parity because it provides evidence on whether or not each ovary has undergone prior expansion. The tracheal network that supplies oxygen to the ovaries includes two large tracheal trunks that branch repeatedly. Nulliparous ovaries include a reserve system to allow for future expansion with tightly coiled tracheoles that appear as knots usually referred to as "skeins." Once blood is imbibed, the ovaries expand as the follicles pass through multiple stages of development. The skeins in the system unwind to service the enlarged ovary and assure that oxygen is available for its greatly enlarged surface area. Once the eggs are laid, the ovary shrinks back down but the skeins do not re-wind. The tracheolar network of a parous mosquito appears as a dense spider web and is used as an accurate indicator of parity. Skeins are present only in host-seeking nulliparous mosquitoes because they have never laid eggs and are looking for their first blood meal.



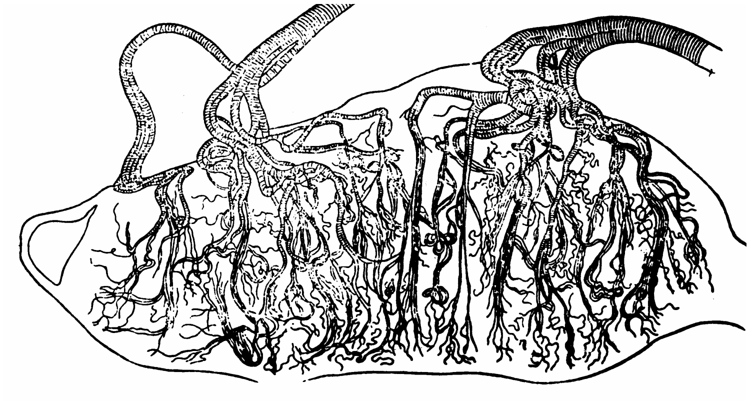


Fig. 1. Ovary of a nulliparous mosquito showing coiled skeins (upper) vs. a parous mosquito showing the network of unwound tracheoles (lower) after Detinova 1962.

Preparing a Template: Parity dissections are best viewed on a microscope slide that is divided into a grid to accept 20 well-aligned ovaries in two rows of 10. This allows a technician to progressively scan from one ovary to the next each under a compound microscope at 100X without having to search for each individual dissection. The easiest way to prepare a suitable grid is to place the microscope slide that receives the ovarian dissections on a black template where the grid has been drawn with a magic marker. The white ovaries stand out against the black background of the template during the dissection process and keeps the two lines of ovaries lined up for later microscopic observation.

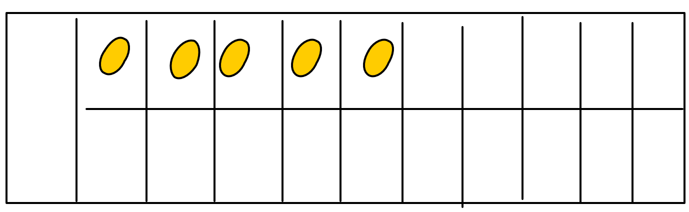


Fig. 2. Useful arrangement of ovarian dissections on microscope slide for parity analysis. Space at the left is used for labeling purposes. Grid can be drawn on a template card using a magic marker.

Dissection Procedure: The two ovaries of a female mosquito form a "Y" with the median oviduct, a tube that exits the insect at the vagina on the terminal segment of the abdomen. Both ovaries are easily drawn out with the tip of the abdomen if the last segment is separated and slowly drawn from the rest of the body. Dissections should be made in physiological saline, positioning a fresh female mosquito with her abdomen just touching a saline drop about the size of a dime. Two dissecting needles made from insect pins are needed to remove the ovaries from the insect and transfer them to the observation slide. Small nicks in the penultimate abdominal segment allow for a weakened area that tears easily when pressure is applied. To accomplish this, hold the female mosquito in place with the needle held in the left hand and make a dorsal and ventral nick with the needle in the right hand taking care to avoid cutting the median oviduct. Then slowly draw the weakened terminal segment away from the body pulling the two ovaries that are still attached into the saline drop on the slide. The ovaries appear as small white oval bodies composed of tiny round ovarioles much like bunch of grapes. The tracheoles are very evident clustered about each ovary and have an elastic property most evident in their torn ends. At least one of the two ovaries should be rinsed briefly in a drop of distilled water to prevent salt crystals from developing in the final preparation. The ovary (or ovaries if both are recovered) should then be transferred to a minute drop of distilled water on position 1 of the template grid and allowed to air dry without additional preparation. The system is repeated for the remaining mosquitoes in the sample that will be used to determine the parous rate. When landing rates are high, 20 specimens should be dissected to determine the parous rate. As the brood ages and the numbers decline, analysis can be based on fewer specimens.

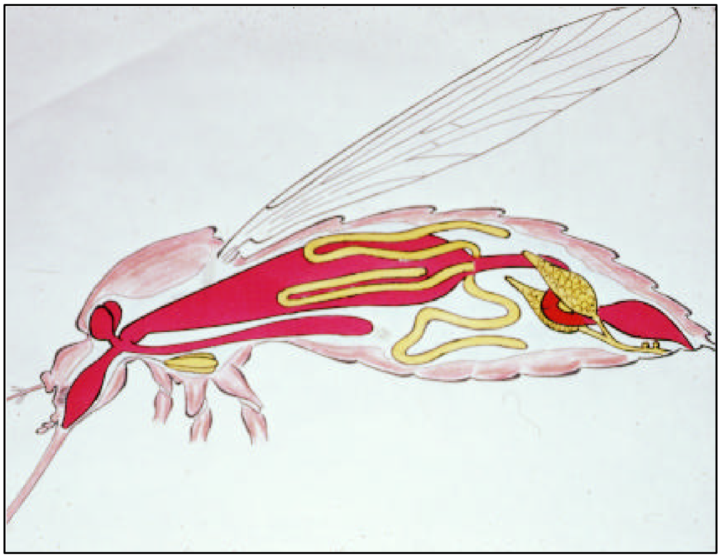


Fig. 3. Location of the ovaries in the abdomen of a female mosquito in relation to the alimentary canal. Ovarian dissection pulls the ovaries out by separating the terminal end of the mosquito from the rest of the body.

Well trained laboratory technicians can complete a full dissection in 30 seconds; inexperienced technicians will take more time. Dissection slides containing ovarian dissections can be labeled and removed from the template as soon as the entire slide has dried. They can be stored in a slide box for later microscopic analysis but can be read as soon as they have dried down.

Microscopic Analysis: Slides for parity analysis should be viewed with a compound microscope at 100X that is equipped with a mechanical stage that allows the reader to scan the upper line of 10 ovaries progressively from left to right. The process is then repeated for the lower row of 10. The presence of distinct skeins in nulliparous ovaries vs. a spider web appearance in parous ovaries is possible because the tracheal network takes on air as the ovaries dry down. Under compound microscopy, the air filled trachea take on a silvery appearance and parity vs. nulliparity is easy to discern. Nulliparity is most evident immediately after a brood has emerged because the skeins are tightly knotted in freshly emerged females. Some unwinding does take place over time in nulliparous mosquitoes that have not successfully obtained blood but experience quickly separates those from specimens that have laid eggs. In general, the ovaries of parous mosquitoes have lost a great deal of elasticity which becomes evident early in the dissection process. A proportion of most parous populations contain females that have one or more retained eggs in ovaries that have shrunk back down. The presence of a retained egg is firm proof of parity but the spider web pattern of the tracheae is used as d