THE COMMONWEALTH OF MASSACHUSETTS
EXECUTIVE OFFICE OF ENERGY AND ENVIRONMENTAL AFFAIRS

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TO: Commissioner John Auerbach (DPH)
Commissioner Laurie Burt (DEP)
Commissioner Scott J. Soares (DAR)
Commissioner Rick Sullivan (DCR)

FROM: State Reclamation and Mosquito Control Board

DATE: December 1, 2010

RE: **Final Report: Aerial adulticiding intervention to diminish risk of Eastern equine encephalitis virus (EEEV), Southeast Massachusetts, 2010**

Introduction

The Department of Agricultural Resources (MDAR), through the State Reclamation and Mosquito Control Board (the Board), in coordination with the Massachusetts Department of Public Health (MA DPH) planned, implemented, and supervised an aerial mosquito control spray operation over a large part of Southeastern (SE) Massachusetts during August 2010. As outlined in the Mosquito-Borne Disease Response Plan, the Board hereby submits its final report regarding the aerial mosquito control spray operation.

The aerial mosquito control spray operation was conducted in response to the elevated risk of mosquito-borne Eastern equine encephalitis virus (EEEV) transmission. Infection by EEEV often leads to a life-threatening disease of human beings as well as elevated morbidity and mortality in certain mammals and birds. Mortality rates in people are expected to approach 50%. Severe life-long abnormalities can occur in nearly 90% of survivors. The goal of the aerial mosquito control spray operation was to cause an immediate and significant reduction in risk of transmission of EEEV to people. Based upon the documented conditions acknowledged to reflect the serious public health threat, a *Certification of Public Health Hazard* was signed on July 31, 2010 by the MA DPH Commissioner John Auerbach. This document certified that the aerial application of was necessary to protect the public in areas of SE Massachusetts where infected adult mosquitoes were most prevalent.

The aerial mosquito control spray operation was scheduled to begin on Wednesday evening of August 4, 2010. Unfavorable weather conditions postponed the spray operation on August 4th until conditions would be conducive for the operation. Accordingly, the aerial mosquito control spray operation began on the next evening Thursday, August 5th. The final applications were made Saturday evening, August 7, 2010.

Description of Aerial Mosquito Control Spray Operation

Prior to the actual operation, 2 aircraft were deployed from the Board's contractor, Dynamic Aviation Company in Virginia, and the product of choice was shipped from Clarke Mosquito Control in Chicago to allow for the required testing of the aerial spray equipment. A two-step calibration and characterization procedure was conducted to ensure that the desired aerial spray application parameters (such as amount of active ingredient (a.i.) dispensed per acre and the optimum droplet size) were achieved for maximum efficacy and to be consistent with the product label.

The testing was conducted by experienced technical personnel of Clarke Mosquito Control, Dynamic Aviation, representatives from MDAR, as well as those from various mosquito control districts such as the Northeastern Massachusetts Mosquito and Wetlands Management District (NMMWMD). In particular, the personnel involved included Fran Krenick, (Good Laboratory Practices Systems Manager, Clarke Mosquito Control), Clarke E. Wood, (Vice President, Clarke Mosquito Control), and Wally Terrill (President of the Northeastern Mosquito Control Association (NMCA)), John Kenney (MDAR representative and former Chair of the Board), and Jack Card (Operations Manager (NMMWMD), and Robyn Januszewski (Biologist, NMMWMD). MDAR pesticide program senior enforcement inspectors Michael McClean and Taryn LaScola conducted a pesticide use observation of the aerial mosquito control operation (See Appendix 3).

The two-step testing was interrupted by unsettled weather conditions and the characterization step could not be could not be held at the general staging location for the operation. Calibration successfully occurred at the Plymouth Municipal Airport on Tuesday, August 3rd. Excessive wind at this location prohibited the testing to take place at this site so it was necessary to locate to another airport in Massachusetts where more favorable and suitable meteorological conditions existed. As a result, characterization of aerial spray equipment successfully occurred on August 4th at the Barnes Airport in Westfield, Massachusetts.

In addition to the essential task of calibration and characterization, the Environmental Police under the command of Colonel Aaron Gross, were requested to be on site for the duration of the operation to keep the base of operation secure in order to address Biosecurity and Homeland Security concerns pertaining storage of aircraft and bulk pesticides.

Once the testing was completed, two (2)-twin turbine Beechcraft King Air (Model A90 numbered N61Q and N78D) commenced the aerial mosquito control operation on August 5th with spray on beginning at 8:16 and 8:15 PM, respectively. The operation ended with spray off at 1:20 AM for both aircraft. On the following evening of August 6th, N61Q and N78D commenced the operation with spray on beginning 8:04 and 8:01 PM, respectively and ending at 1:52 and 1:40 AM, respectively. Both aircraft completed the operation on the last evening of spraying Saturday, August 7th which commenced at 8:01 PM and ending at 10:05 PM.

The aerial mosquito control operation covered a total area encompassing 288,143.20 acres over defined portions of Bristol and Plymouth County as calculated by the navigational flight system of the aircraft. The treated area included all or parts of the following 27 municipalities: Acushnet, Berkley, Bridgewater, Carver, Dartmouth, Duxbury, East Bridgewater, Easton, Fairhaven, Freetown, Halifax, Hanson, Kingston, Lakeville, Marion, Mattapoisett, Middleborough, New Bedford, Norton, Pembroke, Plympton, Plymouth, Raynham, Rochester, Taunton, Wareham, and West Bridgewater.

The aircraft applied the 1,394.84 gallons of Anvil 10 +10 ULV (EPA # 1021-1688-8329), at a rate of 0.62 oz/acre (the maximum allowable amount permitted by the pesticide product label), and at a height of 300 feet above the ground. The aircraft average airspeed ranged from ~ 170.9 - 173.8 mph, and dispensed an aerosol swath width of 750 feet for aircraft N61Q and 1,000 feet for aircraft N78D. In addition to the actual amount of product applied to reduce the mosquito population, 24.81 additional gallons of Anvil 10 +10 ULV were employed for droplet size testing of the 2 aircraft prior to the operation. Thus, the total amount of product consumed for the entire aerial mosquito control spray operation was 1,419.65 gallons.

Anvil 10+10 ULV contains the active ingredients d-phenothrin (sumithrin) and the synergist Piperonyl Butoxide (PBO). This particular product and formulation was the product of choice and selected based on prior interagency assessment.

Weather Conditions

Reported weather conditions during the August 5, 6 and 7, 2010 aerial application ranged from optimal to acceptable. All weather parameters remained within ranges compatible with the pesticide product label. Pesticide labeling for Anvil 10+10 ULV states that Air temperature should be greater than 50 degrees F when conducting all types of applications. These weather conditions, in general, reflected conditions favorable to mosquito activity during the application windows.

On Thursday, 8/5/2010 optimal conditions existed during the entire spray window. Those conditions included temperatures remaining in the low seventies during the application window. Light to moderate winds prevailed during the entire application.

On Friday, 8/6/2010 conditions were optimal at the start of the application window, with temperatures in the low seventies and light winds. In the final two hours of the application acceptable weather conditions existed with temperatures ranging from the mid-fifties to the low sixties with wind speeds reported from calm to five mph.

On Saturday, 8/7/10 weather conditions were acceptable during the application window. Temperatures remained optimal throughout the application with a range from the low 60s to the low 70s. Low to moderate wind speeds were reported at the start of the application. Wind speeds decreased towards the end of the application with reports ranging from three mph to calm conditions.

Results of Aerial Mosquito Control Spray Operation

As designed, the aerial mosquito control spray operation dramatically reduced the populations of mosquitoes in the treated area. The populations of mosquitoes considered to mainly feed upon mammals (and to pose greatest immediate risk to people) were diminished by 90%; overall, mosquito populations in the treated area were reduced by 80%. Although mosquitoes collected prior to the intervention repeatedly were found to harbor EEEV, none sampled immediately after the intervention were infected, nor were those sampled between August 10^{th} -20^{th} .

Entomologists from the Bristol and Plymouth County Mosquito Control Projects reported significant decreases in mosquito abundance in the areas that were treated. Bristol County reported overall reductions for all species at 90.6% on night two (8/6-7, 2010) and 88.3% on night three (8/7/2010). In particular, Bristol County noted that the aerial spraying reduced the target species (mammal biting species) *Coquillettidia perturbans* 87.0% on night two (6-7 Aug 2010) and 89.7% on night three (7 Aug 2010). Plymouth County reported an overall reduction of 80.84% of all mosquito species, including 87.71% reduction of *Coquillettidia perturbans*.

Similarly, the MA DPH arbovirus program field coordinator also reported a significant decrease in mosquito abundance, with overall control of 76.6%. In addition, the aerial application reduced the targeted mammal biting species *Coquillettidia perturbans* by 89.45%. See Summary Table in Appendix 1.

The aerial intervention was also designed to reduce the abundance of adult *Culiseta melanura*, the enzootic vector that cycles EEEV between birds (and occasionally to mammals). Reducing these populations is considered of value in reducing the ability of EEEv to perpetuate in a site. Dramatic decreases in enzootic mosquito abundance post intervention were reported by Bristol County and Plymouth county mosquito control projects. Bristol County documented a reduction of (96.2%) for the 2nd night of spraying and (96.5%) for the 3rd night. Plymouth County documented a reduction of (68.95%) with somewhat less impressive reductions (38.9%) for *Culiseta melanura* reported by MA DPH.

Note: The 2010 MA DPH results pertaining to *Culiseta melanura* control were consistent with those realized from the 2006 aerial application. The difference between the MA DPH and the regional mosquito control projects results may be a function of where each surveillance expert sited its traps. The MA DPH standard surveillance traps are sited in more heavily forested areas containing *Culiseta melanura* habitat. Such sites with dense canopies tend to limit penetration by the pesticide aerosol. Furthermore, continued emergence of young adult *Culiseta melanura* from these sites were reported adding to the overall and sampled populations. Hence, continual emergence or immigration of adults to a treated area can severely encumber the interpretation of efficacy assessments that rely solely upon abundance data. Ultimately, it is clear that Bristol, Plymouth, and DPH all used the same approach and formula to calculate efficacy. Even with variability in some reductions, all of the results reveal that the aerial spray operation resulted in a positive conclusion that overall the absolutes numbers of mosquitoes were significantly reduced in the treated spray zone especially *Coquillettidia perturbans* the targeted species. See Appendix 2 as reported by surveillance specialists.

The aerial mosquito control spray operation achieved the stated goals, these being the immediate and significant reduction of the mosquito population, and the lessening of transmission risk. Because it neither is possible nor desirable to completely and permanently eradicate the mosquito population or to totally eliminate risk of EEEV transmission, the public was advised to remain vigilant in avoiding mosquitoes and to take simple common-sense precautions to prevent mosquito bites for the remainder of the season. As was fully anticipated, although the intervention dramatically reduced EEEV transmission risk, the target mosquito population began to recover soon, thereafter. The intervention afforded the transient results desired; no long-term effects have been noted from this or similar applications elsewhere.

<u>Environmental Monitoring</u>

Environmental Monitoring can confirm the absence or presence of negative impacts to the environment as a result of the aerial mosquito control spray operation. Bees, drinking water supplies, macroinvertebrates, cranberries and pesticide illness surveillance, non-target species/rare or state listed rare species were all monitored and/or evaluated by different state agencies regarding the aerial mosquito control spray operation (**See Appendices 1-7**).

Water Supplies-Department of Environmental Protection

Sumithrin was not detected in any pre or post spray water sample of drinking water in the treated region. Piperonyl Butoxide (PBO) was not detected in any of the pre-spray samples. PBO was detected at very low concentrations in certain post-spray raw water samples as well as in two surface water samples from non-public water supply pond/impoundments, but not in any of the finished drinking water samples. The very low concentrations of PBO detected were below reporting limits (0.1 ug/L) with detections ranging from 0.11 to 0.36 ug/L are far below levels that are considered to be of any public health concern.

Biomonitoring of Macroinvertebrates- Department of Environmental Protection

No acute impacts to aquatic, aerial or terrestrial non-target invertebrate populations were evident subsequent to the aerial spraying of Anvil 10 +10 based upon biomonitoring sampling or in less formal field observations of aerial/terrestrial invertebrate activity. Post-spray samples at each biomonitoring site revealed aquatic communities that were undiminished both in terms of taxonomic richness and their apparent vigor (ability to move rapidly to evade capture). Whereas the post-spray samples in the treated zone had one or two taxa missing or "rare" that had been common in the pre-spray sample this was also observed in the reference waterbody which had three taxa that were "common" in the pre-spray sample and "rare" in the post-spray. Additionally, there was no commonality in the missing/rare taxa among sites. In each of the data sets, the "missing" or "rare" taxon at one site was observed and common at one or more of the other sampling locations.

Biomonitoring samples from the aquatic habitats, and informal field observations of aerial/terrestrial invertebrate activity, are meant to reveal immediate non-target impacts from the intervention. These data do not address questions of potential chronic impacts related to pesticide application. Rather, they attest that efforts taken to minimize the acute impact on non-target invertebrates appear to have been successful.

Bees-Department of Agricultural Resources

In the event aerial adulticiding is necessary in response to threat of EEEV transmission risk, and in accordance with the Board's Operational Response Plan, MDAR performs environmental monitoring of a random selection of honey bee hives in the proximity of proposed application areas to evaluate colony health before and after the spraying of Anvil 10+10 ULV application. Accordingly, the state apiary inspector (Mr. Al Carl) and the seasonal apiary inspector (Mr. Ken Warchol) inspected honey bee colonies located in Bristol and Plymouth Counties. Honey bee colonies were inspected visually to assess their populations and vigor beginning August 2nd and proceeding through August 10th. Of the 57 colonies inspected, 49 were monitored for bee mortality utilizing cotton sheets placed in front of the hives. This environmental monitoring technique was designed to collect and document dead or dying bees.

The state apiary inspectors observed that the bees were storing prodigious amounts of honey derived from the sweet pepper bush (*Clethra alnifolia*). They confirmed that these bushes were nearly at peak of nectar flow during this period, and the inspectors could readily detect the characteristic odor from drying *Clethra* honey. These observations provided evidence of colony health and vigorous bee activity.

Post spray inspections revealed that all colonies exhibited good population strength and vigor for this time of year (early August) both before and after the aerial spray mosquito control operation. In some colonies, worker bees exhibited deformed wings, a sign of a viral infection and an indication of substantial *Varroa* mite populations. State apiary inspectors contended that if honey bees foraging nectar from *Clethra* were exposed to sumithrin in the field, then the foraging population would have diminished and colonies would be less vigorous and/or the cotton sheets would be covered with dead bees. All colonies observed post aerial mosquito control operation appeared vigorous.

Bee colonies were surveyed for population strength, brood diseases, and *Varroa* mites. None of the colonies surveyed experienced any form of population decline that could be attributed to aerial spray mosquito control operation.

Results

After inspection and survey of 57 hives with 49 being assessed using cotton sheets as survey tools to capture dead bees, assess and document record potential spray injury, and after examination of dead bees post aerial application on Monday, Tuesday, and Wednesday (August 9th , 10th, 11th) the state apiary inspectors concluded that no significant mortality was observed. There was 1 hive where corn spray injury was observed in Rochester on 8/09/2010; with 57 dead bees collected from the sheet. All other sites ranged from 5 to 6 dead bees per cotton sheet well within normal hive mortality. The inspection and survey results indicate that there was no significant bee mortality and further no injury could be attributed to the aerial spray mosquito control operation.

Cranberries-Department of Public Health

In response to public health concerns about EEEV risk, Massachusetts conducted wide-area aerial pesticide application (ULV) in parts of southeastern Massachusetts in August 2010. The active ingredient of the pesticide used (Anvil 10+10) is sumithrin, which has a federal food tolerance of 0.01 parts per million (ppm). To ensure that sumithrin residues, if any, would not exceed the food tolerance for cranberries in the spray zone, MDPH Bureau of Environmental Health (BEH) collected cranberry samples from seven areas in southeastern Massachusetts, including one non-treated location outside of the spray area, both pre- and post-spray. Cranberry sample collection was coordinated with the Cape Cod Cranberry Growers Association (CCCGA). Pre-spray samples were collected on August 4, 2010, and post-spray samples on August 10, 2010, or approximately 48-72 hours post spray (spraying conducted over August 6-7, 2010). Each of the 7 locations sampled during both rounds included 5 subsamples that were representative of the bog being sampled. The MDPH Hinton Lab Institute (SLI) held subsamples in refrigeration immediately after collection and before shipping these on dry ice via overnight UPS to Golden Pacific Laboratories, LLC, in Fresno, CA. Chain of custody and shipping manifest documentation were maintained intact. The 5 subsamples per location were composited into one sample per 7 locations per round. Analyses of cranberries were conducted in accordance with EPA, FIFRA, Good Laboratory Practice Standards (GLP); 40 CFR, Part 160 (October, 1989). The analytical method measured sumithrin using liquid chromatography tandem mass spectrometry (LC-MS/MS). The established limit of quantitation (LOQ) is 10 parts per billion (ppb) and the limit of detection (LOD) is 2.0 ppb. No measureable residues of sumithrin were detected in any of the cranberry samples (i.e., both pre- and post samples were all (ND) non-detects, or <2.0 ppb). The cranberry test results demonstrated that there were no impacts to this food product that would jeopardize food safety.

Post-Spray Pesticide-Related Illness Surveillance Plan-Department of Public Health Bureau of Environmental Health (BEH), through the Environmental Toxicology Program (ETP)

While the general population is not expected to experience health impacts at levels of exposure to the amount of Anvil 10+10 ULV used during aerial applications related to combating EEE/WNV, pesticide illness surveillance and recommendations are issued to the general public in order to completely avoid and/or minimize direct exposure to the extent possible during this type of mosquito control operation. The goal of the Post-Spray Pesticide-Related Illness Surveillance Plan is to document reported cases of illness during the aerial operation application period. In addition, the ETP answers health-related questions from the public about the use of the pesticide and health related concerns. The pesticide illness surveillance and recommendations issued by BEH in advance of spraying demonstrated that the majority of the general public heeded advice and recorded very few reported health impacts. Only three (3) health related effects were documented ranging from headache, tiredness, and body aches and notably all were among individuals who reported that they did not take precautions as recommended. Two calls were received August 6th from Carver and one on August 9th from Freetown. None of the reported cases indicated that follow-up with a physician was warranted.

Non-Target Species- MA Division of Fisheries and Wildlife, Natural Heritage & Endangered Species Program

The Lloyd Center for the Environment conducted a pilot light trap sampling program to assess impact of the aerial mosquito control spray operation on non-target species in the Hockomock Wildlife Management Area. The following is a summary of the study to detect non-target effects of mosquito spraying in August 2010.

Introduction

This document summarizes a technical report by Mark Mello, an entomologist at the Lloyd Center for the Environment located in Dartmouth MA.
The study was an effort to detect large scale impacts to insects other than mosquitoes and to help estimate impacts to state protected insects. Because of the last minute nature of this project no appropriate control site could be identified and the decision was made to maximize the sampling effort within the spray zone.

Methods

The powerline corridor bisecting the northwest corner of the spray zone that crosses Route 138 in Easton and extends past the Maple Street extension in West Bridgewater was chosen as the study site due to its lack of canopy cover and its accessibility by vehicle. Ten stations were established along a 2,700-meter segment of the powerline at stanchions supporting the powerlines. The stations are roughly equidistant at approximately 300-meter intervals. The light from the traps illuminated a similar sized circle at each of the stations. Five portable 15 watt quantum ultraviolet traps charged with a killing agent were set prior to dusk and retrieved after sunrise the following morning on two pre- spray nights (August 3 and 4) and two post spray nights (August 7 and 8). The spraying event at this location occurred on August 6.Twenty samples were thus acquired: 10 each from both the pre- and post spray event. Each station was sampled once during the pre- and post spray periods. Dominant vegetation species were recorded within a roughly 30 meter radius from the trap site.

All material collected in the traps was saved and at the Lloyd Center until groups could be sorted into petri dishes. All sorting was completed within a week of sample collection. Mello conducted all the fine sorting and counting to species (macrolepidoptera), family (microlepidoptera and beetles) and order for the remaining insects. All samples have been saved, and selected voucher specimens pinned.

Results

A total of 22,939 specimens were counted and identified to different levels. This represents all the trapped specimens except for some very tiny mites that were not counted in this study. No species listed in the Massachusetts Endangered Species Act were encountered. Fourteen insect orders are represented in the samples (Table 3), the predominant orders being beetles (12,059), true flies (3,357), Moths (2,685), and plant lice (2,584). Detailed analyses are presented on these orders as well as on three additional orders with a mean of twenty or more individuals per trap (ants, caddisflies and mayflies). Minimum temperatures were highly variable, with the two warmest nights occurring pre-spray (63° and 72° F.) and the coolest nights post spray (54° and 57° F.). Maximum temperature ranged from 84° to 90° F. Two statistical tests were applied to the data, an analysis of variance and Chi^{2}ts (variable were conducted on nine major insect groups (those that contained a mean of 20 or more individuals per sample, 400+ individuals). Most groups declined between 47 and 77%. Two orders, true flies and mayflies increased substantially. Table 1 shows the percentage increase or decrease by group.

Group	**Common Name**	**% Change**
Coleoptera	Beetles	-47%
Diptera	True flies	+318%
Lepidoptera	Moths	-64%
Homoptera	Plant hoppers	-73%
Hymenoptera	Ants, wasps	-77%
Trichoptera	Caddisflies	-62%
Ephemeroptera	Mayflies	+147%
Hemiptera	True bugs	-70%
Psocoptera	Psocids (Plant, bark lice)	-63%

Table 1

The significant variation between pre- and post spraying samples for two thirds of the groups of insects tested, although likely due in at least part to the spraying event, is confounded by the lower temperatures during the post spray trap nights. Insect activity is lowered by lower air temperature.

The Virginia chain fern borer moth (protected as a species of Special Concern) would not have been in adult winged phase at the time of the spraying so would not have been susceptible to insecticide. Mayflies and true flies, especially aquatic midges, are well known for their mass emergences, perhaps explaining the great increases in their numbers after spraying occurred.

In general insects are well adapted to periodic, infrequent perturbations to their habitats and populations would likely recover to pre-event numbers in a short period of time. This study should be treated as a pilot study to help design a more robust study that includes greater preparation time in order to improve its design and implementation.

GIS: 2010 Mapping, Assessment, and Analysis

Geographic Mapping Information, Communications, and Coordination

GIS (Geographic Information Systems) increasingly are employed in planning and tracking anti-mosquito interventions. Planning, operations, mapping excluded areas, assessment of results, monitoring, and dissemination and distribution of information to the public all rely on accurate and timely GIS data.

A multi-agency response protocol stipulates that MDAR coordinates compilation of mosquito treatment sensitive areas data layers (no-spray zones). Mosquito treatment sensitive areas data layers include:

- Certified organic farms
- Priority habitats for spray sensitive state-listed rare species
- Surface Water Supply resource areas
- Commercial Fish hatcheries/aquaculture

The data layers are developed by MDAR, DFW & Natural Heritage & Endangered Species Program (NHESP), and Department of Environmental Protection (DEP) within the designated MA DPH delineated spray area into a final GIS data layer. Each Department was aware that spraying could occur up to 500 feet (one half the spray swaths) inside the exclusion areas, and the borders of the exclusion areas were created with this in mind. MDAR, for instance, buffered organic farms by 500 feet. As a result, the spray swath rarely impinged upon organic farm boundaries. The aerosol applied by the aircraft is designed to treat the airspace. The droplets are intended to float in the air column. Most would evaporate before impacting the ground. Hence, relatively little insecticide or carrier should be expected to reach crops or other terrestrial and aquatic environments.

The exchange and vetting of map layers between the various state agencies went relatively smoothly. All Departments posted their layers on the SharePoint folder set up by IT. DEP posted the overall spray area, NHESP posted critical endangered species habitat, DEP posted public water supplies, and DAR posted a layer of organic farms and fresh water aquaculture. NHESP data were edited to remove an exclusion area deemed critical by DPH, a non-registered organic farm was added, small parts of several towns were excluded to help simplify public notification messages, and coastal exclusion areas were created to protect aquaculture and marshes. The compiled layer was examined by GIS staff from all departments, resulting in several corrections and adjustments. Transfer of data and communications with the aviation company were also accomplished without difficulty.

Mapping Products

MDAR GIS provided several maps for use by the media and public. On the day of the first flight a map depicting the planned spray polygon was released to, and broadcast by, the media. Because the map resolution was limited, there was some concern that some viewers might conclude they were outside the spray area when in fact they were inside, or vice versa. On succeeding nights the maps symbolized every town in which spraying might occur. One benefit of this approach was that it corresponded directly and consistently with press releases containing only written lists of affected towns. Although this was a safe approach, it was unsatisfactory insofar as users had less information on whether they were in the planned spray area or not, since most towns were not to be sprayed in their entirely on any given night. Complicating the situation was the fact a particular area might be in the planned spray zone two nights running, because operational factors might prohibit a planned spray area from being completed (as happened on the first night).

On the afternoon before the final night's spraying, MDAR's web master and GIS staff teamed up to produce a prototype web map http://www.mass.gov/agr/spray-map/ . Mosquito control professionals from the Plymouth and Bristol County Mosquito Control Projects were anxious to have this data so that surveillance traps could be appropriately located in order to meet objectives of efficacy protocols. The map, using Google Maps base layers, allows the user to zoom to any area to see whether they are in the planned spray area or not on each night. This map would unquestionably be useful to other monitors, such as MDAR's Apiary Inspector and the companies contracted to provide independent environmental monitoring.

GIS Mapping and Future Aerial Operations

A web map might serve as a primary public information source in future years. It would need to be updated every day. The print messages on the map would be critical to avoid misunderstandings. Whether maps are created for hard copy or for the web, they can, to some extent, be planned long beforehand and in some detail so that they can be revised and disseminated as quickly as possible. The speed with which maps are created could probably be further enhanced. To this end, collaboration between MDAR, DFW, NHESP, DEP, and DPH in the off-season is needed. It must be noted that spray operations are influenced by the local weather conditions. Certain changes in temperature, wind and precipitation may each preempt or postpone such an operation. Hence, maps and plans released even shortly prior to an activity may change, by necessity, on a moment's notice.

Finally, it should be noted that were MDAR to experience the network difficulties that have been common on the fifth floor of 251 Causeway Street for the last several months, MDAR's web master and GIS staff would have to move to a different floor or building (with connectivity to the ENV network) to operate effectively.

Costs of Aerial Mosquito Control Spray Operation

The entire cost of the aerial mosquito control spray operation totaled $869,898 dollars. The following is a breakdown:

Aerial Service	$342,890
Product	$276,832
Environmental Monitoring Study	$ 5,372
Plymouth Mosquito Control Project	$ 132,555
Bristol Mosquito Control Project	$ 111,533
Travel	$ 716
Total	$869,898 dollars

Note: The costs denoted for both Bristol and Plymouth County Mosquito Control Project covered additional costs to respond the elevated risk such as overtime for staffing, fuel for truck mounted ground spray equipment, additional mosquito testing, and exhausting exisiting pesticide stock.

Conclusion of Aerial Mosquito Control Spray Operation

The aerial mosquito control spray operation conducted on August 5-7, 2010 to reduce the risk of Eastern equine encephalitis virus (EEEV) was deemed successful as evidenced by immediate and significant reductions in the abundance and infection rates of the targeted mosquitoes *Coquillettidia perturbans* and *Culiseta melanura* in the treated region. The reduction in risk is likely attributed to the aerial spraying operation. As anticipated, virus continued to circulate at reduced frequency within mosquitoes and birds after the aerial spray operation. Although the intervention markedly diminished risk, it did not (nor was it meant to) completely eliminate risk. One human case was confirmed by the Department of Public Health during the 2010 mosquito season within the Commonwealth. Finally, the absence of a detectable negative impact to the environment as reported after environmental monitoring further attests to the overall success of the aerial spray mosquito control operation.

Appendix 1

Aerial Adulticiding Efficacy Results Summary Table

Who	Species	Non-Treatment		Treatment		% Reduction
	Cattail Mosquito	Pre	Post	Pre	Post	
Matthew Osborne, Field Coordinator DPH Arbovirus Program	*Coquillettidia perturbans*	925	716	2260	184	89.45%
Ellen Bidlack, Entomologist Plymouth County Mosquito Control	*Coquillettidia perturbans*	43	30	420	36	87.71%
Wayne Andrews, Superintendent Bristol County Mosquito Control	*Coquillettidia perturbans*	7	6	9	1	87.0%
Wayne Andrews, Superintendent Bristol County Mosquito Control	*Coquillettidia perturbans*	11	10	128	12	89.7%

Appendix 2 **<u>Aerial Adulticiding Efficacy Results</u>**

EEEV Intervention Efficacy – MA DPH 2010

Species	Control		Treatment	
	Pre	Post	Pre	Post
Overall	1187	916	2678	483
Culiseta melanura	37	34	178	100
Coquillettidia perturbans	925	716	2260	184

Overall control – 76.6%
***Culiseta melanura* control – 38.9%**
***Coquillettidia perturbans* – 89.45%**

Ocherotatus canadensis control – Insufficient Collections
Aedes vexans control – Insufficient collections

Prepared and Reported by Matthew Osborne, Field Coordinator Massachusetts Arbovirus Program, Department of Public Health State Laboratories, Jamaica Plain, MA

EEEV Intervention Efficacy - Bristol County 2010

Species	Night Two (8/6-7/2010)	Night Three (8/7/2010)
Culiseta melanura	96.2%	96.5%
Coquillettidia perturbans	87.0%	89.7%
Aedes vexans	100.0%	60.8%
Ocherotatus canadensis	100.0%	58.3%
Overall reduction all species	90.6%	88.3%

Prepared and Reported by, Wayne Andrews, Medical Entomologist, Superintendent
Bristol County Mosquito Control Project, Taunton, MA

EEEV Intervention Efficacy - Plymouth County 2010

Species	Control (5 traps)		Treatment (4 traps)	
	Pre (4-5 Aug 10)	Post (6-7 Aug 10)	Pre (4-5 Aug 10)	Post (6-7 Aug 10)
Overall	84	92	729	153
Culiseta melanura	19	19	248	77
Coquillettidia perturbans	43	30	420	36
Aedes vexans	2	1	4	2
Ocherotatus canadensis	9	5	2	6

Overall control: 80.84%
***Culiseta melanura*: 68.95%**
***Coquillettidia perturbans*: 87.71%**
Not enough data for *Aedes vexans* and *Ocherotatus canadensis*

Prepared and reported by: Ellen Bidlack, Entomologist, Assistant Superintendent
Plymouth County Mosquito Control Project, Kingston, MA

Appendix 3

Division of Crop and Pest Services-Pesticide Enforcement

As part of an effort to ensure that the aerial mosquito control operation conducted in southeastern Massachusetts was in compliance with the State Pesticide Control Act and regulations pertaining to pesticide use in Massachusetts, senior pesticide enforcement personnel from the Department of Agricultural Resources, Division of Crop and Pest Services were present during the mixing/loading activities prior to operation.

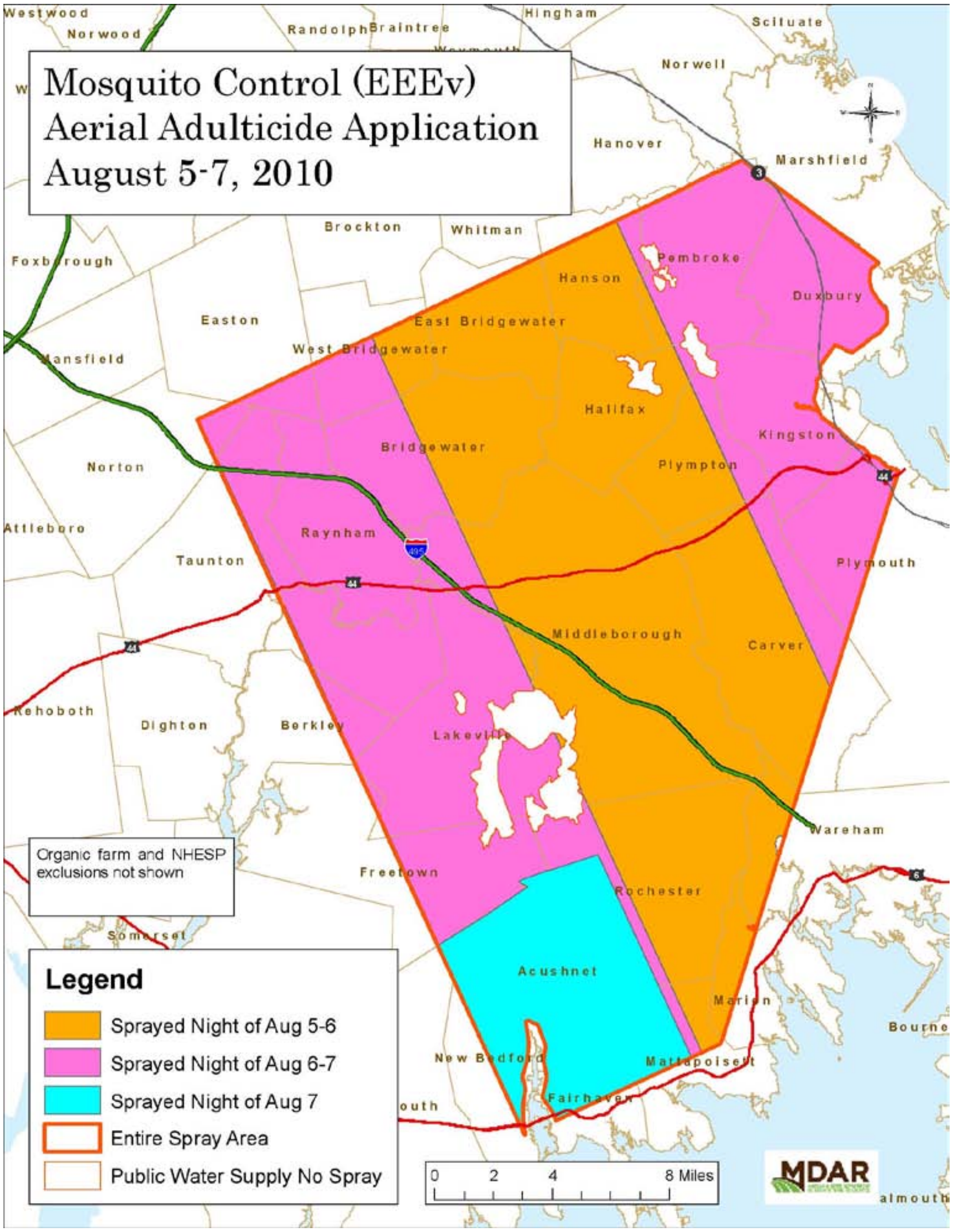
On August 5, 2010 Inspector's Michael McClean and Taryn LaScola arrived at the base of operation in Plymouth, MA to conduct a use observation of the aerial mosquito control operation authorized to reduce the risk of EEEV. The inspectors noted that the aerial service, Dynamic Aviation, located in Bridgewater, Virginia had two aircraft on site be readied for the operation. The two pilots employed by Dynamic Aviation held Massachusetts pesticide credentials confirmed and documented valid and current.

Anvil 10 + 10 ULV (EPA Reg #1021-1688-8329) was the product selected for the operation. Two other personnel on site employed by Clarke Mosquito Control products held a Massachusetts Pesticide credential which were confirmed and documented a valid and current These employees were responsible for handling and loading Anvil 10+10 ULV.

At approximately 6:15 P.M., loading activities started to prepare aircraft for spraying. During the first flight the plane(s) were carrying a total of 180 gallons of product. This was equivalent to three (3) fifty-five (55) gallon drums and one partial drum. The handlers were wearing long sleeves, long pants, boots, gloves and goggles.

A tank mix sample MWM100805-2 MWM was collected of Anvil 10 +10 ULV to confirm that the product was formulated correctly. Laboratory analysis results confirmed that the product was chemically satisfactory. At approximately 7:50 P.M., both aircraft took off from the airport to ferrying to position to commence spray operation. After the first flight mission, Dynamic Aviation landed to refill and fuel aircraft for second flight mission that evening. Senior enforcement personnel were satisfied with their inspection noting no violations during their inspection.

Appendix 4


Mosquito Control (EEEv)
Aerial Adulticide Application
August 5-7, 2010
Organic farm and NHESP exclusions not shown
Legend
Sprayed Night of Aug 5-6
Sprayed Night of Aug 6-7
Sprayed Night of Aug 7
Entire Spray Area
Public Water Supply No Spray
0 2 4 8 Miles
MDAR
Westwood
Norwood
Randolph
Braintree
Hingham
Scituate
Norwell
Hanover
Marshfield
Brockton
Whitman
Foxborough
Pembroke
Hanson
Duxbury
Easton
East Bridgewater
West Bridgewater
Mansfield
Halifax
Kingston
Bridgewater
Norton
Plympton
Attleboro
Raynham
Taunton
Plymouth
Middleborough
Carver
Rehoboth
Dighton
Berkley
Lakeville
Wareham
Freetown
Rochester
Somerset
Acushnet
Marion
Bourne
New Bedford
Mattapoisett
Fairhaven

Appendix 5

REPORT

Water Monitoring Results Associated With Aerial Pesticide Spraying for Mosquitoes in August 2010

Massachusetts Department of Environmental Protection
Office of Research and Standards
Boston, MA

August 2010

MEMORANDUM

TO: Gary Gonyea, BRP
FROM: Michael Hutcheson, PhD., MPH, Office of Research and Standards
cc: Carol Rowan West, Director, ORS; Diane Manganaro, ORS; Jonathan Hobill, SERO; Michael Quink, SERO; Leslie O'Shea, SERO; Dave Terry, DWP; Damon Guterman, DWP; Dennis Dunn, DWM
DATE: August 13, 2010

RE: Water Monitoring Results Associated With Aerial Pesticide Spraying for Mosquitoes In August 2010

INTRODUCTION

Wide-area aerial spraying with the insecticide Anvil 10+10 containing the active ingredient Sumithrin (chemical name phenothrin) was performed in southeastern Massachusetts on August 5-7, 2010. The intent of this effort was to kill adult mosquitoes potentially carrying Eastern Equine Encephalitis (EEE) The water of those surface water bodies serving as sources of drinking water for public water supplies plus some additional surface water bodies was sampled prior to any spraying, and at two times after spraying. Finished water samples were also taken and analyzed.

METHODS

Details of application procedures and flight path information are available from the Massachusetts Department of Agricultural Resources. Flight crews were instructed to spray up to the edges of surface water bodies.

Water sampling took place several days before spraying to serve as a documentation of background conditions. The field sampling protocol for water samples called for sampling at 3 and 24 hours after spraying. Because of operational and logistical constraints, actual times of sampling after spraying were not exactly as prescribed. Sampling in the locations took place approximately 3 and 24-hours after the last aerial application in the week's program; not after a specific location had been sprayed. Therefore individual lake samples were taken at varying times since spraying took place over the three day period. More than likely, actual times from spraying in a location until actual samples were taken was many hours to several days longer than the nomimal 3 or 24 hours assigned to the reading[1].

The water was analyzed for Sumithrin and the insect synergist, piperonyl butoxide (PBO). Analytical work was performed at the Massachusetts Pesticide Analysis Laboratory at the University of Massachusetts at Amherst. Results were provided to MassDEP in report form on August 10 and 11, 2010 (attached as Appendix A). Details of the analytical procedure employed can be obtained from the laboratory.

Current toxicity information on Sumithrin and PBO was gathered from the US EPA's Registration Eligibility Documents for these compounds (U.S. EPA 2006, 2008).

[1] It should be possible to determine the actual times that individual location samples were taken since spraying in that location by cross referencing flight logs, GPS records and sampling time information. This has not been done for this report because of time and resource constraints and our wish to get these results and interpretation released.

RESULTS and DISCUSSION

The analytical results are summarized in Table 1. The reported method detection limits were 0.02 ug/L for both compounds and the Limits of Quantitation for both were 0.1 ug/L. Quality control results for both compounds were within acceptable limits for accuracy. No data were provided on analyses of replicate samples to indicate the level of precision of the analyses.

No Sumithrin was detected either before or after spraying in any water sample.

PBO was not detected in any samples prior to spraying. It was detected at low sub ug/L (parts per billion) concentrations in some raw water samples at the 3- hours after application designated time, although it was not detected at that time in any finished water samples. It was detected in several of the raw water samples at these very low concentrations 24-hours after the completion of the last spraying event, but was not detected in any finished water samples.
These results are consistent with both our past projections of likely water concentrations of these compounds in surface water sources after aerial spraying with Sumithrin in southeastern Massachusetts in 2006 (Hutcheson 2005) and with water quality monitoring results after that spray operation. In fact, measured water concentrations were less than predicted. In the 2005 modeled exercise, calculations were performed assuming the worst case that aerial flights inadvertently overflew surface water bodies, thereby depositing the herbicide directly on the water. As noted earlier, operational procedures in both 2006 and 2010 instructed pilots to cease applications at the edges of water bodies. We projected that initial surface water concentrations after mixing could have reached sub- ug/L concentrations for the Sumithrin and PBO.

None of the reported PBO concentrations approached concentrations that would be of any health concern from either short or long-term exposures. The applicable drinking water concentration limits for Sumithrin and PBO are presented in Table 2 (Drinking Water Equivalent Levels, DWELs). These limits were derived for this report from oral reference doses (RfD) contained in the U.S. EPA's Registration Eligibility Decisions for both these compounds which were produced after our 2005 analysis. The current toxicity values are lower than those presented in our 2005 report (Hutcheson 2005), indicating that the compounds are presently judged to be more toxic than had previously been the view. However, the drinking water concentrations derived from these more current RfDs are still relatively high and orders of magnitude greater than measured concentrations of PBO and Limits of Quantitation for Sumithrin (if there were any Sumithrin present below the Limit of Quantitation); indicating no public health concern since concentrations are nowhere near approaching exposure limits guidance values.

Table 1. Summarized Surface Water Sampling Results for Sumithrin and PBO

Sample Location Code	**Plant/Location**	**Town**	**PBO concentration,ug/L**			**Sumithrin concentration, ug/L**		
			Bkrd	**+3 hr**	**+24 hr**	**Bkrd**	**+3hr**	**+24 hr**
PWS Surface Water Supply Samples								
Ab/Rock 001 finished	Great Sandy Bottom WTP	Abington/Rockland	ND	ND	ND	ND	ND	ND
Ab/Rock-002 raw	Great Sandy Bottom WTP	Abington/Rockland	ND	0.11	0.11	ND	ND	ND
Ab/Rock-003 finished	Hingham St. WTP	Abington/Rockland	ND			ND		
Ab/Rock-004 raw	Hingham St. WTP	Abington/Rockland	ND			ND		
4201000 raw	Quittacas WTP	New Bedford	ND			ND		
4201000 finished	Quittacas WTP	New Bedford	ND			ND		
4044000 Silver Lake raw	Silver Lake WTP	Brockton	ND	0.12	0.12	ND	ND	ND
4044000 Silver Lake finished	Silver Lake WTP	Brockton	ND	ND	ND	ND	ND	ND
021 Elders Pond raw	Elders Pond WTP	Taunton	ND	<LOQ	< LOQ	ND	ND	ND
022 24” finished	Elders Pond WTP	Taunton	ND	ND	ND	ND	ND	ND
10370 finished	Quittacas WTP	New Bedford		ND	ND		ND	ND
10400 raw	Quittacas WTP	New Bedford		<LOQ	ND		ND	ND
Non-PWS Surface Water Samples from ponds & impoundments								
TSL100808-1	control sample[1], Park Pond	Medway		ND			ND	
01A	Pudding Brook Impoundment	Pembroke		ND			ND	
02A	Jones River Impoundment	Kingston		<LOQ			ND	
03A	Sampson Pond	Carver		<LOQ			ND	
04A	Nemasket River	Middleborough		ND			ND	
05A	Skeeter Pond	Bridgewater		<LOQ			ND	
JS1	New Bedford Reservoir	Acushnet		0.36			ND	
JS3	Snipatuit Pond	Middleborough		0.31			ND	
JS5	Lake Rico	Taunton		<LOQ			ND	
JS7	Lake Sabatia	Taunton		<LOQ			ND	

NOTES: BKRD = background sample; ND=not detected. The limit of detection is 0.02 ug/L, and the limit of quantitation (LOQ) is 0.1 ug/L.

All results noted as < LOQ detected something at less than the LOQ value.

Table 2. Toxicity Data and Drinking Water Exposure Limits for Anvil 10+10 Active Ingredient and Additive

COMPOUND	Duration	RfD, mg/kg/d	Source	DWEL*
Sumithrin	chronic	0.007	US EPA 2008	250 ug/L
	acute	0.03	US EPA 2008	300 ug/L
PBO	chronic	0.016	US EPA 2006	5,600 ug/L
	acute	6.3	US EPA 2006	31,500 ug/L

* DWEL – Drinking Water Equivalent Level. chronic DWELs calculated for an average 70 kg person consuming 2L water per day; acute DWELs calculated for a child of 10 kg consuming 1L/d.

CONCLUSIONS

- Water quality monitoring of surface waters and finished drinking water samples after wide-area spraying with Anvil 10+10 containing Sumithrin and PBO did not produce any detectable concentrations of Sumithrin in these resources.
- PBO was detected at very low sub-ug/L (parts per billion) concentrations: far below levels of any public health concern.

REFERENCES

Hutcheson, M. S. 2005. Evaluation of potential human health risks from Sumithrin exposure through drinking water from surface waters sources inadvertently sprayed during mosquito control operations. Memorandum dated September 29, 2005 to Dave Terry, MassDEP Drinking Water Program. Massachusetts Department of Environmental Protection, Office of Research and Standards. Boston, MA.

U.S. EPA. 2006. Registration Eligibility Decision for Piperonyl Butoxide (PBO). List B, Case No. 2525. Report EPA 738-R-06-005 from Office of Prevention, Pesticides and Toxic Substances .June 2006. Washington DC

U.S. EPA. 2008. Registration Eligibility Decision for d-Phenothrin. List A. Case No. 0426. Report from Office of Prevention, Pesticides and Toxic Substances. September 2008. Washington, DC.

APPENDIX A

Laboratory Water Sampling Reports From the Massachusetts Pesticide Analysis Laboratory at the University of Massachusetts at Amherst

Massachusetts Pesticide Analysis Laboratory

Morrill 1 N427A
639 North
Pleasant Street
University of Massachusetts
Amherst, MA
01003-0230
Phone: 413-545-4369

Massachusetts Department of Environmental Protection

Report of Analysis

Sumithrin/PBO Water Analysis

Reviewed and Approved by:

Jeffery J. Doherty
Laboratory Manager

Massachusetts Pesticide Analysis Laboratory

Report Date: 8/10/10
Project: DEP
Container: 1 L amber glass
Preservation: 4°C storage
Matrix: water

Sampled: 8/3/10
Received: 8/4/10
Extracted: 8/5/10
Analyzed: 8/5/10
Analyst: SAM/JJD

RESULTS Background samples

Sample	PBO	Sumithrin
Ab/Rock 001	ND	ND
Ab/Rock-002	ND	ND
Ab/Rock-003	ND	ND
Ab/Rock-004	ND	ND
4201000 raw	ND	ND
4201000 finished	ND	ND
4044000 Silver Lake raw	ND	ND
4044000 Silver Lake finished	ND	ND
021 Elders Pond	ND	ND
022 24” finished	ND	ND

Notes:
ND = not detected. The limit of detection is 0.02 μg/L, and the limit of quantitation (LOQ) is 0.1 μg/L.

QC Results

Parameter	Recovery	QC Limits
PBO (0.1μg/L)	95.5 %	60% -120 %
Sumithrin (0.1μg/L)	87.8 %	60% - 120 %

Massachusetts Pesticide Analysis Laboratory

Report Date: 8/10/10
Project: DEP
Container: 1 L amber glass
8/9/10
Preservation: 4^{o}C storage
8/9/10
Matrix: water
SAM/JJD

Sampled: 8/8/10
Received: 8/8/10
Extracted:
Analyzed:
Analyst:

RESULTS 3 hour sample collection		
Sample	**PBO**	**Sumithrin**
TSL100808-1	ND	ND
104000 raw	**detected< LOQ**	ND
10370 finished	ND	ND
Silver Lake raw	**0.12ug/L**	ND
Silver Lake finished	ND	ND
021 Elders Pond	**detected <LOQ**	ND
022 24" finished	ND	ND
01 A	ND	ND
02 A	**detected< LOQ**	ND
03 A	**detected< LOQ**	ND
04 A	ND	ND
05 A	**detected <LOQ**	ND
JS1	**0.36ug/L**	ND
JS3	**0.31ug/L**	ND
JS5	**detected <LOQ**	ND
JS7	**detected<LOQ**	ND
Ab/Rock 001 finished	ND	ND
Ab/Rock 002 raw	**0.11ug/L**	ND

Notes:
ND = not detected. The limit of detection is 0.02 µg/L, and the limit of quantitation (LOQ) is 0.1 µg/L.

QC Results		
Parameter	**Recovery**	**QC Limits**
PBO (0.1µg/L)	96.7 %	60% -120 %
Sumithrin (0.1µg/L)	90.7 %	60% - 120 %

Massachusetts Pesticide Analysis Laboratory

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639 North Pleasant
Street
University of Massachusetts
Amherst, MA 01003-0230
Phone: 413-545-4369

Massachusetts Department of Environmental Protection

Report of Analysis

Sumithrin/PBO Water Analysis

Reviewed and Approved by:

Jeffery J. Doherty
Laboratory Manager

Massachusetts Pesticide Analysis Laboratory

Report Date: 8/11/10
Project: DEP
Container: 1 L amber glass
Preservation: 4°C storage
Matrix: water

Sampled: 8/9/10
Received: 8/9/10
Extracted: 8/10/10
Analyzed: 8/10/10
Analyst: SAM/JJD

RESULTS 24 hour collection samples

Sample	**PBO**	**Sumithrin**
Ab/Rock 001	ND	ND
Ab/Rock 002 raw	**0.11ug/L**	ND
10370	ND	ND
104000	ND	ND
Silver Lake raw	**0.12ug/L**	ND
Silver Lake finished	ND	ND
021 Elders Pond	**detected <LOQ**	ND
022 24" finished	ND	ND

Notes:
ND = not detected. The limit of quantitation (LOQ) is 0.1 µg/L.
The limit of detection (LOD) is 0.02 µg/L.

QC Results

Parameter	**Recovery**	**QC Limits**
PBO (0.1µg/L)	104 %	60% -120 %
Sumithrin (0.1µg/L)	98.4 %	60% - 120 %

Appendix 6

Technical Memorandum

Biomonitoring Report on the 2010 Aerial Insecticide Spraying Over Southeastern Massachusetts

Robert M. Nuzzo
2 September 2010

Massachusetts Department of Environmental Protection

Division of Watershed Management

Worcester, MA

CN 308.0

Contents

List of Tables

Introduction

The Commonwealth of Massachusetts's 2010 operational plan for managing mosquito-borne diseases (Corte-Real, et al. 2010) outlines the roles of the Commonwealth's environmental agencies in circumstances where a public health emergency related to Eastern Equine Encephalitis virus (EEEv) or West Nile virus (WNv) infections lead to aerial application of insecticide to control the disease vectors (mosquitoes). By early August 2010 the Massachusetts Department of Public Health (DPH) had determined that risk factors (including EEEv titers and vector population densities) in several southeastern Massachusetts towns indicated that aerial insecticide application was necessary to reduce the risk of EEEv infection there. In accordance with the operational plan, MassDEP/Division of Watershed Management (DWM) biologists conducted biological monitoring to coincide with aerial spraying of the insecticide Anvil 10 + 10 over this region of the state. The biological monitoring was designed as a semiquantitative evaluation of acute impairment to aquatic life caused by the aerial insecticide application, as indicated by the macroinvertebrate communities in lentic waterbodies in the treatment zone.

Methods

Samples of aquatic macroinvertebrates were collected from four waterbodies within the anticipated aerial spray zone and one waterbody outside that area. Sampling activities were scheduled to occur prior to and following the aerial spraying event. Pre-spray sampling took place at the selected waterbodies from 2-4 August 2010. Aerial spraying took place on the evenings of 5, 6, and 7 August 2010, and follow-up macroinvertebrate sampling at the selected waterbodies was completed on 11, 12, and 16 August 2010. Table 1 lists the sampling locations and collection dates.

Table 1. Sampling location descriptions and sampling dates.

Waterbody	Location Designator and Description	Pre-spray sample date	Post-spray sample date
Nemasket River	"S1": downstream from Nemasket Street and upstream from Oliver Mill, Middleborough, MA	2 Aug. 2010	12 Aug. 2010
Snipatuit Pond	"S2": littoral zone adjacent to boat launch, Neck Rd., Rochester, MA	3 Aug. 2010	11 Aug. 2010
Skeeter Mill Pond	"S3": littoral zone along northern edge; access from Water St., Bridgewater, MA	3 Aug. 2010	12 Aug. 2010
Elm St. Impoundment (Jones River)	"S4": littoral zone along southern edge; access from park at Elm St., Kingston, MA	4 Aug. 2010	11 Aug. 2010
Park Pond	"R1": littoral zone along northern edge, east of inlet; Choate Park, Medway, MA	2 Aug. 2010	16 Aug. 2010

The samples were composites of five one-meter sweeps of a kick-net (457 mm x 229 mm mouth; 500 µm mesh opening) through littoral zone vegetation from three separate locations within the waterbody. Samples were picked "live" (i.e., unpreserved). Voucher specimens of each potentially unique taxon were retained and relative abundances were qualitatively rated as rare, common, or hyperabundant. Specimens were identified to family in the lab and life stage (larva/nymph, pupa, adult) present was noted. Since many families may have been represented by more than one species, an attempt was made to estimate the number of species present. Estimates were based on the taxonomist's sight-recognition of the genus/species or recognition of morphological differences that indicated two or more unique species were present (without pursuing the identification further).

While not formally part of the biomonitoring effort, DWM biologists made an effort to take note of aerial/terrestrial invertebrates active at the sampling sites on the dates visited. These observations were recorded on the field sheets, except for pre-spray visits to S3 (Skeeter Mill Pond) and S4 (Elm St. Impoundment).

Results

The lists of aquatic macroinvertebrate taxa collected, relative abundances, species estimates, and life stage(s) in samples bracketing the aerial spraying of Anvil 10 + 10 are presented in the Appendix, Tables A1-A5. The aquatic macroinvertebrate communities collected from all biomonitoring locations following the aerial spraying event were found to be vital and robust: In every case, observed behaviors and activity of the specimens in the sample were regarded as normal, and the number of taxa detected was higher in follow-up samples than in the initial samples (Table 2). Each of the sites had one to three taxa post-spray that were missing or "rare" despite having been common in the pre-spray samples.

Table 2. Families present and species estimates. Summary of the number of aquatic macroinvertebrate families represented, and estimated number of species present, in each sample before and after aerial spraying of Anvil 10 + 10.

	S1-pre	S1-post	S2-pre	S2-post	S3-pre	S3-post	S4-pre	S4-post	R1-pre	R1-post
Families	20	28	14	24	10	20	28	31	21	27
Est. spp.	≥ 27	≥ 38	≥ 21	≥ 36	≥ 15	≥ 28	≥ 36	≥ 44	≥ 26	≥ 38

Location S1—Nemasket River

Sampling at this location took place in the emergent and submergent vegetation of the lentic region between the Nemasket Street bridge and the head of the mill races at the Oliver Mill Park in Middleborough. The predominant aquatic plants were *Potamogeton* spp., *Sparganium* sp., *Myriophyllum* sp., and *Elodea* sp.

The presence of adult damselflies (Odonata: Zygoptera) was recorded on the field sheets at the time of the pre-spray sampling. At the time of the post-spray follow-up sampling bumble bees (Hymenoptera: Apidae) were prominent as they visited the numerous clover flowers in the park lawn near the river. Also recorded were damselflies, an adult beetle (Coleoptera), an adult tipulid (Diptera: Tipulidae), and a spider.

With no decrease in taxonomic richness between pre-spray and post-spray samples (Table A1), there is no evidence from the aquatic macroinvertebrate community data to suggest acute harm associated with the aerial spraying. Naucorids (Hemiptera: Naucoridae), however, were common in the pre-spray samples but undetected post-spray.

Location S2—Snipatuit Pond

Littoral zone vegetation in the sampled areas of this pond included *Pontederia cordata*, *Juncus* sp., *Utricularia* sp., *Nymphaea* sp., and *Sagittaria* sp. Adult damselflies were observed during the initial visit. During the resampling visit the following aerial/terrestrial invertebrates were recorded: bumble bees, adults of dragonflies (Odonata: Anisoptera) in the families Libellulidae and Aeshnidae, damselflies, an adult chrysomelid beetle (Coleoptera: Chrysomelidae), and spiders (at least three distinct species).

With no decrease in taxonomic richness between pre-spray and post-spray samples (Table A2), there is no evidence from the aquatic macroinvertebrate community data to suggest acute harm associated with the aerial spraying. The snail family Planorbidae was the only group common in the pre-spray samples that was reduced to "rare" in the follow-up samples.

Location S3—Skeeter Mill Pond

Nymphaea sp. and *Cabomba caroliniana* were by far the dominant littoral zone plants. Also present were *Nuphar variegata*, *Ceratophyllum demersum*, *Brassenia schreberi,* and *Pontederia cordata.* Observations of aerial/terrestrial invertebrates were neglected on the pre-spray visit. Post-spray, adult dragonflies (≥ 2 spp.), adult damselflies, numerous aphids (Hemiptera: Aphididae) on lily pads, adult true flies (Diptera), grasshoppers (Orthoptera), and an adult weevil (Coleoptera: Curculionidae) were all observed at the site.

With no decrease in taxonomic richness between pre-spray and post-spray samples (Table A3), there is no evidence from the aquatic macroinvertebrate community data to suggest acute harm associated with the aerial spraying. Among the taxa captured in pre-spray samples, only larvae of the family Corduliidae were not detected in the post-spray samples.

Location S4—Elm St. Impoundment of the Jones River

Sampling points here were accessed through a small park on Elm Street. The littoral zone vegetation was dominated by *Utricularia* sp., *Myriophyllum* sp., *Pontederia cordata*, *Sparganium* sp., and *Nymphaea* sp. Observations of aerial/terrestrial invertebrates were not included with the field data from the pre-spray sampling. Recorded post-spray were bumble bees, a larval ladybird beetle (Coleoptera: Coccinellidae), adult beetle (other than Coccinellidae), and spiders.

With no decrease in taxonomic richness between pre-spray and post-spray samples (Table A1), there is no evidence from the aquatic macroinvertebrate community data to suggest acute harm associated with the aerial spraying. Two taxa that were common in the pre-spray samples were not detected in the post-spray collections: Crambidae (Lepidoptera) and Pleidae (Hemiptera).

Location R1—Park Pond

This location was chosen because it was well outside of the area expected to be covered by the aerial spraying of Anvil 10 + 10, and thus could serve as a reference. Samples were collected in dense beds of *Peltandra virginica* with some *Pontederia cordata* present as well. Young-of-year centrarchids (probably pumpkinseeds) were captured in abundance in the sweeps, giving the impression that these beds of vegetation are important refugia for them. Activity of dragonfly adults, bumble bees, and two distinct species of spiders at this location were noted during the preliminary sampling. On the follow-up visit only spiders were recorded.

The list of aquatic macroinvertebrates collected from Park Pond on dates bracketing the aerial spraying in southeastern Massachusetts is shown in Table A5. The results from this location are very similar to the other sample locations in that more taxa were revealed in the follow-up samples than in the initial samples. Furthermore, there were three groups rated as “common” in the initial samples that were “rare” in the later samples.

Conclusions

Follow-up sampling at each of the biomonitoring sites inside the aerial spray zone was completed within seven days of the aerial spray application. The post-spray samples in every case revealed aquatic communities that were undiminished, both in terms of taxonomic richness and the apparent vigor (ability to move rapidly, attempts to evade capture, etc.) of the specimens captured. The post-spray samples from each biomonitoring site in the treatment zone had one or two taxa missing or “rare” that had been common in its respective companion pre-spray sample. The reference waterbody in Medway had three taxa that were common in the preliminary sample and rare in the follow-up sample.

Reduction of a population common in the community prior to insecticide treatment to “rare” or “missing” following treatment was suggested as a possible indicator of an impact on the community. In each of these data sets, however, the “missing” or “rare” taxon was observed and common at one or more of the other biomonitoring locations following the aerial spray application (Table 3). These observed reductions, therefore, cannot be causally linked to the treatment.

It is apparent from the biomonitoring samples from the aquatic habitats, and from less formal associated field observations of aerial/terrestrial invertebrate activity, that acute impacts from the aerial spraying of Anvil 10 + 10 were not evident in either case. These data do not address, nor were they intended to address, questions of chronic impacts related to pesticide application. Rather, they demonstrate that efforts taken to minimize the acute impact on non-target invertebrates appear to have been successful.

This conclusion is further supported by the water sample analysis reported by Hutcheson (2010). Among the waterbodies where macroinvertebrate samples were collected, the only quantifiable concentration of the Anvil 10 + 10 active ingredients 3h after the aerial spray application (Table 4) was found in Snipatuit Pond at less than one part per billion (0.31 µg/L of the synergist, piperonyl butoxide).

Table 3. Presence/absence, before/after comparison. This table indicates the presence of macroinvertebrate populations before and after the aerial spraying of the insecticide (Anvil 10 + 10) in instances where populations were reduced or missing in post-spray samples (highlighted).

	S1 before/after	S2 before/after	S3 before/after	S4 before/after	R1 before/after
Viviparidae					X/R
Planorbidae		X/R	R/0	X/X	0/R
Pisidiidae		0/R		X/X	X/R
Asellidae	0/R	X/X		X/X	X/R
Corduliidae		0/X	X/0	X/X	X/X
Naucoridae	X/0			X/X	
Pleidae		0/X	X/X	X/0	X/X
Crambidae	X/X	R/0	0/R	X/0	

X = common; R = rare; 0 = not detected

Table 4. Water sample concentrations. Results of analysis for Anvil 10 + 10 ingredients, Sumithrin and Piperonyl Butoxide (PBO), in water samples collected from biomonitoring waterbodies at 3h after aerial spraying. (From Hutcheson 2010.)

Waterbody[1]	Town	PBO	Sumithrin
Park Pond[2] (R1)	Medway	ND[3]	ND
Nemasket River (S1)	Middleborough	ND	ND
Snipatuit Pond (S2)	Rochester	0.31 µg/L	ND
Skeeter Mill Pond (S3)	Bridgewater	<LOQ	ND
Jones River Impoundment (S4)	Kingston	<LOQ[4]	ND

[1] See Table 1 for location descriptions.
[2] Reference waterbody, located outside spray area.
[3,4] NOTES: ND=not detected. The limit of detection is 0.02 µg/L, and the limit of quantitation (LOQ) is 0.1 µg/L. All results noted as < LOQ detected something at less than the LOQ value.

Literature Cited

Corte-Real, L., A. Carroll, G. Gonyea, M.S. Buffone. 2010. 2010 operational response plan to reduce the risk of mosquito-borne disease in Massachusetts. Massachusetts Dept. of Agricultural Resources. Boston, MA. 76 pp.

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APPENDIX

Table A1. Taxa List for Nemasket River, Middleborough, MA. List of aquatic macroinvertebrate taxa in samples before and after aerial spraying of Anvil 10 + 10. Collection dates were 2 August (S1-pre) and 12 August 2010 (S1-post).

Taxa	S1-pre	S1-post
Mollusca, Gastropoda		
Hydrobiidae	R	R
Ancylidae		R
Lymnaeidae		X
Physidae		R
Annelida, Oligochaeta		
Naididae		X
Annelida, Hirudinea		
Glossiphoniidae	R	
Crustacea, Isopoda		
Asellidae		R
Crustacea, Amphipoda		
Gammaridae	X	X
Hyalellidae	X	X
Acariformes		
Hydrachnidia	X	X (2 spp.)
Insecta, Ephemeroptera		
Baetidae	X	X (3 spp.)
Heptageniidae		X
Insecta, Odonata		
Aeshnidae	X (2 spp.)	X (≥ 1 sp.)
Calopterygidae	X	X
Coenagrionidae		X
Gomphidae		
Insecta, Hemiptera		
Belostomatidae		X
Gerridae	R	
Mesoveliidae	R	R
Naucoridae	X	
Nepidae		R
Notonectidae		R
Megaloptera		
Corydalidae	R	R
Insecta, Trichoptera		
Hydropsychidae		R
Leptoceridae	R	R
Polycentropodidae	R	X
Insecta, Lepidoptera		
Cambridae	X	X
Noctuidae	R	
Insecta, Coleoptera		
Elmidae		A (2 spp.)
Gyrinidae		X

Table A1. (Continued.)

Taxa	S1-pre	S1-post
Insecta, Diptera		
Chironomidae	X (≥ 7 spp.)	X (≥ 7 spp.)
Culicidae	R (L+P)	R
Sciomyzidae	R	
Simuliidae	R (L+P)	R
Families	20	28
Estimated spp.	≥ 27	≥ 38

X—Larval/nymphal stage (the default, except Hemiptera may be nymph or adult) present and common in sample.
A—Adult specimen(s) present; common unless otherwise indicated.
P—Pupal specimen(s) present; common unless otherwise indicated.
L—Larval/nymphal specimen(s) present in addition to other life stage indicated.
R—Present but relatively rare in sample.

Table A2. Taxa List for Snipatuit Pond, Rochester, MA. List of aquatic macroinvertebrate taxa in samples collected before and after aerial spraying with Anvil 10 + 10. Collection dates were 3 August (S2-pre) and 11 August 2010 (S2-post).

Taxa	S2-pre	S2-post
Mollusca, Gastropoda		
Hydrobiidae		X
Ancylidae		X
Physidae		X
Planorbidae	X	R
Mollusca, Pelecypoda		
Pisidiidae		R
Annelida, Oligochaeta		
Naididae		R (2 spp.)
Annelida, Hirudinea		
Glossiphoniidae		R (2 spp.)
Crustacea, Isopoda		
Asellidae	X	X
Crustacea, Amphipoda		
Crangonyctidae	R	
Hyalellidae	X	X
Acariformes		
Hydrachnidia	X (≥3 spp.)	X (≥3 spp.)
Insecta, Ephemeroptera		
Baetidae	X (2 spp.)	X
Caenidae	X	X
Insecta, Odonata		
Aeshnidae		X
Coenagrionidae	X	X
Corduliidae		X
Lestidae		X
Libellulidae	X	X
Insecta, Hemiptera		
Belostomatidae	R	R
Corixidae		R
Nepidae	R	
Notonectidae		R
Pleidae		X
Insecta, Trichoptera		
Leptoceridae		R
Insecta, Lepidoptera		
Crambidae	R	
Ceratopogonidae		X
Chironomidae	X (≥ 5 spp.)	X (≥ 9 spp.)
Culicidae	R	
Families	14	24
Estimated spp.	≥ 21	≥ 36

X—Larval/nymphal stage (the default, except Hemiptera may be nymph or adult) present and common in sample.
R—Present but relatively rare in sample.

Table A3. Taxa List for Skeeter Mill Pond, Bridgewater, MA. List of aquatic macroinvertebrate taxa in samples before and after aerial spraying of Anvil 10 + 10. Collection dates were 3 August (S3-pre) and 12 August 2010 (S3-post).

Taxa	S3-pre	S3-post
Mollusca, Gastropoda		
Hydrobiidae		R
Ancylidae		X
Physidae		R
Planorbidae	R	
Annelida, Oligochaeta		
Naididae		X (2 spp.)
Glossiphoniidae		R
Hyalellidae	X	X
Acariformes		
Hydrachnidia	X (≥3 spp.)	X (≥3 spp.)
Insecta, Ephemeroptera		
Caenidae		X
Insecta, Odonata		
Coenagrionidae	X	X
Corduliidae	X	
Lestidae	X	X
Libellulidae	X	X
Insecta, Hemiptera		
Belostomatidae		R
Corixidae		R
Hebridae	R	R
Pleidae	X	X
Insecta, Trichoptera		
Leptoceridae		R (2 spp.)
Insecta, Lepidoptera		
Crambidae		R
Insecta, Diptera		
Ceratopogonidae		X
Chaoboridae		R
Chironomidae	X (≥ 4 spp.)	X (≥ 5 spp.)
Families	10	20
Estimated spp.	≥ 15	≥ 28

X—Larval/nymphal stage (the default, except Hemiptera may be nymph or adult) present and common in sample.
R—Present but relatively rare in sample.

Table A4. Taxa List for Elm Street Impoundment of the Jones River, Kingston, MA. List of aquatic macroinvertebrate taxa in samples before and after aerial spraying of Anvil 10 + 10. Collection dates were 4 August (S4-pre) and 11 August 2010 (S4-post).

Taxa	S4-pre	S4-post
Mollusca, Gastropoda		
Ancylidae	X	X
Lymnaeidae		X
Physidae		X
Planorbidae	X (3 spp.)	X
Mollusca, Pelecypoda		
Pisidiidae	X	X
Annelida, Oligochaeta		
Naididae	R (2 spp.)	R (2 spp.)
Tubificidae	R	R
Annelida, Hirudinea		
Glossiphoniidae	X (2 spp.)	X (2 spp.)
Erpobdellidae	X (2 spp.)	X
Crustacea, Isopoda		
Asellidae	X	X
Crustacea, Amphipoda		
Gammaridae		X
Hyalellidae	X	X
Crustacea, Decapoda		
Cambaridae	R	
Acariformes		
Hydrachnidia	X (≥ 3 spp.)	X (≥3 spp.)
Insecta, Ephemeroptera		
Baetidae		X
Caenidae	X	X
Insecta, Odonata		
Aeshnidae		X (2 spp.)
Coenagrionidae	X	X
Corduliidae	X	X
Gomphidae		X
Lestidae		X
Libellulidae		
Insecta, Hemiptera		
Belostomatidae	R	R
Corixidae	R	
Naucoridae	X	X
Nepidae		R
Notonectidae	X	X
Pleidae	X	
Veliidae		R
Insecta, Trichoptera		
Dipseudopsidae	R	
Leptoceridae	R	X
Polycentropodidae		X

Table A4. (Continued.)

Taxa	S4-pre	S4-post
Insecta, Lepidoptera		
Crambidae	X	
Insecta, Coleoptera		
Dytiscidae	R	
Elmidae	R (A)	A
Haliplidae	A	L + A (2 spp.)
Hydrophilidae	R (A)	
Insecta, Diptera		
Chironomidae	X (≥ 2 sp.)	X (≥ 8 spp.)
Psychodidae	R (P)	
Sciomyzidae		R
Total Families	28	31
Estimated spp.	≥ 36	≥ 44

X—Larval/nymphal stage (the default, except Hemiptera may be nymph or adult) present and common in sample.
A—Adult specimen(s) present; common unless otherwise indicated.
P—Pupal specimen(s) present; common unless otherwise indicated.
R—Present but relatively rare in sample.

Table A5. Taxa List for Park Pond, Medway, MA—out-of-spray-area reference sample. List of aquatic macroinvertebrate taxa in samples before and after date of aerial spraying of Anvil 10 + 10 over southeastern Massachusetts. Collection dates were 2 August (R1-pre) and 16 August 2010 (R1-post).

Taxa	R1-pre	R1-post
Mollusca, Gastropoda		
Viviparidae	X	R
Physidae	R	R
Planorbidae		R
Mollusca, Pelecypoda		
Pisidiidae	X	R
Annelida, Oligochaeta		
Naididae		R (2 spp.)
Annelida, Hirudinea		
Glossiphoniidae	R	
Erpobdellidae	R	R
Crustacea, Isopoda		
Asellidae	X	R
Crustacea, Amphipoda		
Hyalellidae	X	X
Crustacea, Decapoda		
Cambaridae	R	
Acariformes		
Hydrachnidia	X	X (≥2 spp.)
Insecta, Ephemeroptera		
Baetidae		X
Caenidae	X	X
Insecta, Odonata		
Coenagrionidae	X	X
Corduliidae	X	X
Gomphidae	X	
Libellulidae	X	X (2 spp.)
Insecta, Hemiptera		
Belostomatidae	R	R
Gerridae		R
Macroveliidae		R
Naucoridae		
Pleidae	X	X
Veliidae		R
Insecta, Megaloptera		
Corydalidae	R	R
Sialidae		R
Insecta, Trichoptera		
Leptoceridae	R	
Polycentropodidae	R	
Insecta, Coleoptera		R (A)
Haliplidae		A

Table A5. (Continued.)

Taxa	R1-pre	R1-post
Scirtidae	R	
Insecta, Diptera		
Ceratopogonidae		X
Canacidae		R
Chironomidae	X (≥ 6 spp.)	X (≥ 9 spp.)
Sciomyzidae		R
Families	21	27
Estimated spp.	≥ 26	≥ 38

X—Larval/nymphal stage (the default, except Hemiptera may be nymph or adult) present and common in sample.
A—Adult specimen(s) present; common unless otherwise indicated.
R—Present but relatively rare in sample.

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PILOT LIGHT TRAP SAMPLING PROGRAM TO ASSESS IMPACTS OF AERIAL SPRAYING OF ANVIL 10 + 10 FOR MOSQUITO CONTROL ON NON-TARGET SPECIES IN THE HOCKOMOCK SWAMP

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ABSTRACT

The seasonally early appearance of Eastern Equine Encephalitis (EEE)-bearing mosquitoes and the occurrence of the disease in both humans and horses triggered a widespread aerial spray operation across 27 southeastern Massachusetts towns with the adulticide ANVIL on August 5-7, 2010. The Lloyd Center was contracted to conduct a pilot pre- and post spray sampling regime within the Hockomock Swamp along a section of powerlines in the northwest corner of the swamp in order to document the the impact of the aerial spray operation on nocturnal Lepidoptera and other night-flying insects active pre- and post spray.

A total of 22,939 arthropods, all but 14 spiders being insects, were documented from portable quantum ultraviolet light traps operated at ten stations (five each sampled on two consecutive nights) both pre- and post spray. Fourteen insect orders are represented in the samples, the predominant orders being Coleoptera (12,059), Diptera (3,357), Lepidoptera (2,685), and Homoptera (2,584). No species listed in the Massachusetts Endangered Species Act were encountered.

Variation (two-way ANOVA) due to the spray event was significant at p=0.01 for Diptera, microlepidoptera, macrolepidoptera, Homoptera, Hymenoptera and Trichoptera; but sampling block variation was significant (p=0.01) for aquatic and terrestrial Coleoptera, Diptera, macrolepidoptera, Homoptera and Trichoptera. Wide variation in the minimum temperatures on each of the four sampling nights and microhabitat differences confound the effects of the spray event itself. However at least the microlepidoptera, which did not vary significantly between sampling nights but did decline significantly post spray appeared to be negatively impacted by the aerial application of ANVIL. Suggestions are made on future studies that would minimize sources of variation so that the impacts of the aerial application of ANVIL can be quantified.

INTRODUCTION

The detection of Eastern Equine Encephalitis (EEE) bearing mosquitoes relatively early in the summer season combined with its confirmation in at least one horse and one human case triggered a widespread aerial spray operation using the insecticide, ANVIL and ANVIL II (T. Simmons, pers. com.) across all or part of 27 towns in southeastern Massachusetts on August 5-7, 2010 (Figure 1). The last widespread aerial spraying of insecticide targeting EEE-carrying mosquitoes in southeastern Massachusetts occurred in 2006 (ANVIL), and prior to that, in 1990 (malathion). The active ingredients in ANVIL are the synthetic pyrethroid, sumithrin (10%), and the synergist, piperonyl butoxide (10%) (=ANVIL 10 + 10). The latter agent serves to enhance the lethal effects of sumithrin on insects. These active ingredients decompose within four hours in sunlight (Central MA Mosquito Control Project). Dilution in open water (3 feet deep) rapidly drops concentration levels below toxicity for insects (Coats, 2003). ANVIL application levels during this spray operation were 0.62 fluid ounces per acre, which is 0.0036 lbs active agent per acre (Buffone, October 20, 2010).

A cursory search for relevant articles documenting effects of ANVIL on non-target insects, particularly nocturnal Lepidoptera and other nocturnal flying insects that would be sampled by ultraviolet light traps produced numerous non-scientific reports and articles tauting either the dire effects of aerial spraying or the lack thereof, nearly always with no references to specific scientific documents backing their claims. Although much scientific work has been done on pesticide threats to non-target animals, I found few that were specific for nocturnal Lepidoptera and other nocturnal flying insects. The following discussion excerpts results from four of the more recent references that were located, but is by no means meant to be a synoptic review of the literature.

Jensen et al. (1999) tested pyrethroids and organophosphate insecticides in a California wetland and found that the applications produced no reduction in abundance or biomass of aquatic macroinvertebrates. Flying insect abundance was significantly reduced, but the numbers rebounded 48 hours after application. Boyce et al. (2007) tested EverGreen, a pyrethrin insecticide synergized with piperonyl butoxide, which "..found no effect of spraying on nontarget sentinel species including dragonflies (*Sympetrum corruptum*), spiders (*Argiope aurantia*), butterflies (*Colias eurytheme*), and honeybees (*Apis mellifera*). In contrast, significantly higher diversity and numbers of nontarget arthropods were found on ground tarps placed in sprayed versus unsprayed areas. All of the dead nontarget species were small-bodied arthropods as opposed to the large-bodied sentinels that were not affected." Breidenbaugh (2008) tested the effects of aerial spray technologies using NALED on non-target insects at the Parris Island Marine Corps Recruit Depot in South Carolina as part of his dissertation thesis. Using malaise trap in 2003, he found that 3-4 of the 12 dominant taxa, all Dipterans were significantly reduced post application, however, t-tests of the pre- and post application on the Shannon-Weaver diversity indices were not significantly different. In 2005, data from pan traps similarly showed reductions in only a quarter of the dominant taxa, and no significant difference between the Shannon-Weaver diversity indices. Breidenbaugh did note that microlepidoptera, which comprised 5-6% of the collections and were therefore not statistically analyzed, nevertheless were reduced in number post spraying. Schleier & Peterson (2010) conducted field tests on crickets with NALED and permethrin, finding no significant differences in mortality between spray and control samples. From this they concluded that these insecticides "...most likely neither will result in population impacts on medium- to large-bodied insects".

Each of the aforementioned references include extensive literature citations, but these have not been throughly reviewed for this paper. Rather, the above cited papers reflect studies that were conducted primarily on field surveys testing the affects of aerial spraying of ultra low volume of insecticides on non-target insects, and using ANVIL or similar pyrethrenoid insecticides. No studies were found that specifically tested non-target nocturnal Lepidoptera, except for the Breidenbaugh (2008) reference to incidental catch (5-6% of all insects) of microlepidoptera in malaise and pan traps. Therefore, in order to obtain baseline data on the nocturnal insect fauna potentially impacted by early August aerial spraying of ANVIL in an extensive wetland system, namely the Hockomock Swamp, the Lloyd Center was contracted to perform the following survey.

METHODS

The powerline corridor bisecting the northwest corner of the spray zone that crosses Route 138 in Easton and extends past the Maple Street extension in West Bridgewater was chosen as the study site due to its lack of canopy, and thus expected uniformity in spray reaching the trap sites, and its accessibility by vehicle (Figure 2). Ten stations were established along a 2,700-meter segment of the powerline at stanchions supporting the powerlines (Figure 3; Table 1). The stations are roughly equidistant at approximately 300-meter intervals. The stanchions as well as the dirt access road are slightly elevated above the otherwise wetland habitat that courses the length of this section of the powerlines due to the importation of artificial fill. The areas immediately under the stanchions were either devoid of vegetation, or colonized by low-growing grasses and forbs. Thus the light from the traps illuminated a similar sized circle at each of the stations. Five portable 15 watt quantum ultraviolet traps charged with ethyl acetate were set prior to dusk and retrieved after sunrise the following morning on two pre- spray nights (August 3 and 4) and two post spray nights (August 7 and 8). The spraying event at this location occurred on the night of August 6/7 (Figure 2). Trap nights are identified by the date the traps were set, i.e., traps operated the evening of August 3 through the morning of August 4 would be identified as being the August 3 trap night. Because of the last minute nature of this project and difficult logistical rearrangement of staff conducting other projects, no appropriate control site could be identified, and the decision was made to maximize the sampling effort within the spray zone.

Stations were identified as A – J, but Stations A – E were alternately interspersed with Stations F – J so that each of these two "Station Blocks" alternated trap stations between each other (Figure 3). In this way the entire length of the powerline study area was sampled each night. Also, this sampling regime minimized the impact of habitat variability across this powerline segment. Twenty samples were thus acquired: 10 each from both the pre- and post spray event. Each station was sampled once during the pre- and post spray periods. Photographs of each station are contained in Appendix I.

Table 2 lists the dominant or co-dominant vegetation present at each station as well as other species observed within a roughly 30 meter radius from the trap site. There is some station to station variability, but generally the dominant and co-dominant plants were Autumn olive, buckthorn, red maple, gray birch, little bluestem grass or Phragmites. The artificial fill brought in to construct the dirt access road and the base support for the stanchions has created habitat primarily for non-native invasive species. The intervening wetlands, with the exception of some Phragmites patches, consist of primarily native plants, including Virginia chain fern, which supports a colony of the MESA-listed *Papaipema stenoceli*s (Special Concern).

The light traps were operated by Lloyd Center staff: Research Technician, Everett Booth, and Research Associate, Jamie Bogart. All material collected in the traps was emptied into plastic tubs and refrigerated at the Lloyd Center until the following groups could be rough sorted into petri dishes by Everett Booth: macrolepidoptera; microlepidoptera, Trichoptera, Coleoptera and other insects greater than 5 mm in length. All rough sorting was completed within a week of sample collection. As each sample was finished with rough sorting, beetles and larger non-Lepidopteran specimens were pre-served in alcohol. Lepidoptera, Trichoptera, and the remaining assortment of smaller Coleoptera and other insect orders were placed in covered petri dishes, labeled, taped together by sample and placed in the freezer. Mello conducted all the fine sorting and counting to species (macrolepidoptera), family (microlepidoptera and beetles) and order for the remaining insects. All samples have been saved, and selected voucher specimens pinned.

Nomenclature for Lepidoptera follows (Hodges 1983), except for the Geometridae (Scoble 1999) and Noctuoidea (Lafontaine & Schmidt, 2010). Nomenclature for Coleoptera families follows Downe & Arnett (1996), and the remaining Orders follow Arnett (1997).

RESULTS

Overview

A total of 22,939 specimens were counted and identified to the taxon level indicated in Methods, all but 14 spiders being insects (Appendix II). This represents all the trapped specimens except for some very tiny mites that were not counted in this study. No species listed in the Massachusetts Endangered Species Act were encountered. Fourteen insect orders are represented in the samples (Table 3), the pre-dominant orders being Coleoptera (12,059), Diptera (3,357), Lepidoptera (2,685), and Homoptera (2,584). Detailed analyses are presented on these orders as well as on three additional orders with a mean of twenty or more individuals per trap (Hymenoptera, Trichoptera and Ephemeroptera). Minimum temperatures at the local Easton, MA weather station were highly variable, with the two warmest nights occurring pre-spray (63° and 72° F.) and the coolest nights post spray (54° and 57° F.). This difference likely had an effect on insect activity pre- and post spray. Maximum temperature ranged from 84° to 90° F. and was likely much less of a factor. Because no control station(s) with similar habitat and temperature regime could be established within the time period between notification of this project and the project inception, interpretation of the following statistical results are speculative.

Results of ANOVA and Chi^2 tests on major insect groups.

Two-way ANOVA tests (variable A – pre- and post spray samples; variable B – station blocks, the sample stations per trap night, A-E and F-J) were conducted on nine major insect groups (those that contained a mean of 20 or more individuals per sample, 400+ individuals total. The first variable (A) compares all ten pre- versus all ten post spraying samples (Table 4). Variable B compares variation within each night's set of five samples, whose variation could include sample location (microhabitat differences), temperature variation, variation in spray reaching the ground, and/or variation in emergence. The variance of the means between pre- and post spraying samples was not significant at p=0.05 for aquatic Coleoptera, Diptera, microlepidoptera, macrolepidoptera, Trichoptera or Ephemeroptera, but it was significant for terrestrial Coleoptera, Homoptera and Hymenoptera. Thus, fore the latter three groups, the ANOVA results may skew the results towards masking significant variation.

The pre- – post spraying source of variation (A) was significant at p=0.05 for all insect groups except aquatic Coleoptera and Ephemeroptera; however, the variation was not significant at p=0.01 for terrestrial Coleoptera. The sources of variation among the sample blocks (B) was significant at p=0.05 for all groups except microlepidoptera and Ephemeroptera; however the variation was not significant at p=0.01 for Hymenoptera. None of the insect groups demonstrated significant variation from A X B interaction, suggesting that habitat composition and spray levels reaching the ground were relatively homogeneous across the samples.

The significant variation between sample blocks for most of the groups most likely reflects in part, the variation in temperature on each of the nights (minimum temperatures of 63, 72, 54 and 57 F. respectively). Because each “station block” actually consisted of every other sample rather than five adjacent samples, microhabitat variation, while apparently significant between some of the samples, would be minimized when comparing the blocks of samples.

The significant variation between pre- and post spraying samples for two-thirds of the groups of insects tested, although likely due in at least part to the spraying event, is confounded by the lower temperatures during the post spray trap nights. Newly emerging individuals, particularly observed for the Diptera and the Ephemeroptera also affect the results, however it is unclear in which direction. It is probably more than a coincidence, however, that the two groups that did not show significant variation at p=0.05 are aquatic and/or have aquatic nymphs or larvae, and may have benefited by dilution of the spray that reached open water (e-mail comments from Mark Buffone, October 20). Microlepidoptera and Hymenoptera both had significant variation between pre- and post spray samples at p=0.01, but station blocks variation was not significant even at p=0.05. This suggests that the reduction in number post spray was primarily due to the spraying event itself. Based on the results of these ANOVA tests, it is difficult to evaluate the role of temperature differences, which were dramatic for minimum temperature each night (station block differences), relative to the spraying event in assessing the reduction in number of individuals in the remaining insect groups.

Chi^2 tests were conducted on the data from each station block sampled on each date in order to test for homogeneity of the samples within station blocks. The calculated Chi^2 value was compared to p=0.01 with 3 degrees of freedom because the “Expected” values were derived from the five data points within each block on each sample date (Table 5). The within block variation among samples was significant for all blocks except: macrolepidoptera (A-E on both dates; F-J on Aug. 8), Hymenoptera

(A-E on Aug. 8 only), and Trichoptera (A-E on Aug. 3 only). Thus the within block stations differed significantly for most of the groups tested, indicating significant microhabitat differences and reflecting some macrohabitat differences that were evident from the dominant vegetation differences at some of the stations (Table 2) despite the general appearance of overall homogeneity of the study site.

Taxon Group Results

Aquatic Coleoptera. Coleoptera were the most abundant group of insects present (12,059), and over 80% of these (9,803) were from nine aquatic families (Table 3). Two-thirds of these were Hydrophilidae (water scavenger beetles). Scirtidae (marsh beetles) was the second-most abundant aquatic family at 2,266 individuals. The Hydrophilidae were significantly more abundant in Station Block F – J on August 4 (pre-spray), particularly at Stations H (1,250) and J (1,437). The Scirtidae were significantly more abundant in Station Block A-E pre-spray (735) than post spray (33), however the F-J Station Block had a much smaller reduction in numbers (816 pre-; 681 post). Because these two families comprise more than 90% of the aquatic beetle fauna, the effects of the spraying, temperature, and habitat variability on these two families account for most of the statistical variability for the aquatic Coleoptera. Aquatic Coleoptera were one of the few groups for which the source of variation due to the pre- and post spray event statistically (Table 4) was not significant. As previously mentioned, this could be due to either the diluting effect of the wetlands once the spray reached water, and/or the microhabitat consisting of dense vegetation (for the Scirtidae) found in the aquatic habitat along the powerline.

Terrestrial Coleoptera. Carabidae (ground beetles) comprised more than half (1,149 of 2,155) of the terrestrial Coleoptera captured during this study. Variation for terrestrial Coleoptera due to station blocks was significant at p= .01, but the variation due to pre- post spray was significant at p = .05 but not at p =.01 despite what appear to be dramatic declines pre and post spray for each station block (Table 3), particularly for the Carabidae and the third-most abundant group, the Coccinellidae (ladybird beetles). However, half of all the Carabidae were from one pre-spray sample (Station J), which also had the highest post-spray number (69) of any station. Thus, this single station, is driving the statistical effect of the F-J station block, thus elevating the effects of station block variation relative to pre-post spray variation. Of the ten stations, Station J likely contains microhabitat that is the most favorable for Carabidae. Staphylinidae, the second most abundant terrestrial Coleoptera family (399) produced dramatically different numbers by station bloc (A-E: 28 pre, 25 post; F-J: 175 pre, 178 post). Although the number of individuals per station also peaked at Station J, the differences were less than half the next most abundant stations, and the Staphilinids appeared to be unaffected by the aerial spray when comparing results within each station block.

Diptera. The 3,357 Diptera counted in this study may be undercounted, as there were many tiny and/or newly emerged individuals in the samples. It is possible that additional similar individuals may have been destroyed by the larger organisms collected in the traps. The ANOVA test shows significant sources of variation at p=0.01 both between pre and post spray and between station blocks.

Microlepidoptera. Of the 2,685 Lepidoptera (all moths) collected, the majority (2,018) belonged to the families of generally small to minute moths known as microlepidoptera. All but 108 were identified to family. Coleophoridae (493), Gelechiidae (426), Tortricidae (406) and Pyralidae (345) contained the bulk of 17 families of microlepidoptera that were documented. The ANOVA test shows significant variation at p=0.01 between the pre- and post spray event, but no significant difference between station blocks at p=0.05. This suggests that the aerial spraying did have a negative effect on this taxon group, as the affects of temperature difference, if significant on this group should be reflected in a significant source of variation for the station blocks. Chi^2 analysis showed that variation among samples within station blocks was not significant at p=0.05, further supporting the conclusion that the greatest source of variation between pre- and post spray was the effects of the spray itself.

Macrolepidoptera. Six hundred sixty-seven macrolepidoptera (the “larger”-sized and less primitive families of moths) representing 123 species were captured in the traps. Although species richness is robust, the number of individuals is very low for summer light trapping, when this total number of macro-moths might be expected in a single trap. The low number reflects a generally early season emergence for most of the spring and summer moth fauna observed at study sites for other projects in 2010, as well as a generally lower diversity and number of individuals seen in wetlands. The high proportion of non-native invasive plants, especially the autumn olive and buckthorn, which are larval hostplants for significantly fewer macrolepidopteran species than native shrubs (records from Robinson, et al. 2002), may also have depressed the number and diversity of macrolepidoptera in the samples. The only species to show up in double-digit numbers in any sample was the lichen-feeding, *Crambidia pallida* (Arctiinae), which had a total of 171 individuals. The next highest individual total was 32 for the wetland species, *Capsula oblonga* (Noctuinae), whose larval hostplants include bulrush and cattail (Robinson, et al. 2002).

Variation was significant at p=0.01 for both the pre- and post spray event and the station blocks. This was largely driven by the relative high numbers in the Aug. 4 pre-spray block relative to any of the other blocks. Also, *C. pallida* is a small species that is a similar size or smaller that many of the Pyralidae and some of the Tortricidae, and thus may suffer similar deleterious effects due to the spray event that the microlepidoptera seem to have suffered.

Homoptera. The 2,484 Homoptera captured were not counted to family, however, 75% or more were Cicadellidae (leafhoppers). Cercopidae (spittlebugs), Flattidae (planthoppers) and Membracidae (treehoppers) comprised the bulk of the remaining Homopterans. Variation was significant at p=0.01 for both the pre- and post spray event and the station blocks. There was a large reduction in number pre- and post spray for each station block (A-E: 619 pre-. 68 post; F-J: 1410 pre-, 487 post). It is not possible, statistically to quantify the effects of temperature as opposed to spray effects, but the discrepancy appears to be too great to be attributable to temperature alone.

Hymenoptera. Seven hundred forty-five winged Hymenoptera were captured in this study, including Ichneumonidae, Branconidae, Diapriidae and especially winged Formicidae. An additional 33 wingless ants were captured as well, and some of these may have been winged when trapped but subsequently lost/shed their wings prior to processing the samples. Variation was significant at p=0.05 for both the pre- and post spray event, and the station blocks, but not significant at the p=0.01 level for the station blocks. The statistical influence of nuptial ant flights, which usually last no more than a day from any given colony, is a confounding factor in interpreting these results.

Trichoptera. The 548 caddisflies collected during this study included microtrichopteran families such as Hydroptilidae. Variation was significant at p=0.01 for both the pre- and post spray event and the station blocks. As with the other taxonomic groups with this pattern of variation, it is difficult to evaluate the role of temperature versus the spray, but the exceeding low number on the night following the spraying (25 at A-E; August 7) relative to the pre-spray August 3 data (137) is suggestive of an impact caused by the spray event (Table 3; Appendix II).

Ephemeroptera. The 434 mayflies that were captured during this study appeared to be overwhelmingly from a single species in the family, Tricorythidae (=Leptohyphidae in Marshall, 2006). Variation was not significant at p=0.01 for either the pre- and post spray event or the station blocks. The short adult lifespan coupled with the possible lack of toxic levels of ANVIL in the water that might affect nymphs that are ready to eclose may explain the apparent lack of impact of the spray event on this taxon. Chi^2 analysis within Station blocks resulted in significant variation at p=0.01 for all four dates, suggesting that the influence of particular stations, particularly stations A and J (Appendix II) may be driving the variation for mayfly numbers during this study.

DISCUSSION

There is a generally statistically significant (Table 4) and graphically obvious (Figure 4) drop in the number of insects within most of the predominant taxonomic groups collected by light trap following the August 6 aerial spray event. Weather, in the form of the significantly different minimum temperatures that occurred on each of the survey nights, however, makes interpretation of this drop in numbers difficult to parse between temperature effects and spray impact. Also, the variability among stations within each sampling block was higher than we would have expected (Table 5), given the appearance of at least superficial homogeneity of macrohabitat along the powerline coursing through a red maple swamp.

ANOVA analysis on the microlepidoptera, detailed under the results section, where the source of variation due to station blocks (which includes temperature variation) was not statistically significant but the pre- post variation was, suggests that microlepidoptera were negatively impacted by the spray event. This is consistent with results found by Breidenbough (2008). Our data is also consistent with the study by Boyce et al. (2007) that concluded that negative impacts occurred on small bodied arthropods but not on larger species. It appears that Homoptera and Trichoptera also suffered drastic reductions in numbers, but the significance of this reduction is not necessarily borne out in the ANOVA analysis. Given the small size of species in the microlepidoptera and Homoptera, it would be reasonable to expect that doses of ANVIL that are lethal to mosquitoes would be lethal to these taxa as well.

Larval stages of Lepidoptera were not surveyed, thus latent effects from larval loss due to spraying would not appear in light trap samples until the following generation. A light trap survey at Myles Standish State Forest the year following the malathion aerial spray operation, however, was inconclusive (Mello, 1992). Larval mortality immediately post spray should be documented, as it is those species that would be most at risk to long-term harm.

Even if one were to assume that the drop in numbers on August 7 (post spray) was primarily due to the aerial spraying itself, the subsequent rebound on August 8 suggests that a one time spray event may cause significant knockdown, but rebound resumes shortly thereafter (Figure 4), which is consisted with previously cited studies. This can be explained by staggered and/or extended emergence periods for many species. In the short term, recolonization due to emigration from adjacent unsprayed areas is only a minor factor in rebound numbers given the widespread extent of the operation, as emigration would occur primarily along the margins of the spray zone – a very small percentage of the acreage sprayed.. Ephemeroptera and Formicidae (Hymenoptera), which have either very short adult lifespan and thus synchronized emergence flight (mayflies), or synchronous nuptial flights (ants) may suffer dramatic losses should a spraying event occur during these flights. Repeated spray operations in the same area would also likely have significant negative impacts on any of the taxa, such as microlepidoptera, vulnerable to ANVIL.

Three Odonata and four Lepidoptera species listed listed in MESA have been documented within, or in the immediate vicinity of the Hockomock Swamp during the past two decades. The Odonates include one damselfly (*Enallagma laterale*) and two dragonflies *Somatoclora linearis* and *S. kennedyi*), the adult flight period of *S. linearis* falling within the time frame of the aerial spray operation. The aquatic nymphs of these species are not likely to be negatively affected by the spray due to the effects of dilution (Coats, 2003). Adult *linearis* may be too large to be killed by the spray, but possibly caged experiments using similar sized species on unlisted *Somatochlora* or other dragonfly species should be undertaken, Schleier III and Peterson (2010) notwithstanding. Neither the one butterfly (*Callophrys hesseli*) nor the three moths (*Lithophane viridipallens*, *Papaipema stenocelis* and *P. sulphurata*) are in the adult stage during the month of August. The two *Papaipema* species, however, fly from mid September to early October and thus potentially could be impacted by a September spray event. Larval stages are not likely impacted, as the two *Papaipema* are stem borers and the other two species would have pupated by August.

Design and implementation of this study was hampered by the short notice (July 31) that a survey was requested and the first sample date (August 3). A suitable study site was found (open canopy powerline cut) that was reasonably homogeneous in order that replicate samples could be taken without running traps at exactly the same site on consecutive nights. The within block variability on any given night indicates that the five trap stations per night was closer to the minimum rather than the maximum number of samples needed per night to obtain statistically useful data. However, no suitable control site was identified that matched the powerline study site. Temperature variability, emergence differences and subtle microhabitat differences (as borne out in this study) work against using a remote site as a control. Ideally unsprayed control strip(s) should be established within the spray zone of any future study site. Lastly, no measurements were made of the spray concentrations reaching the ground. Because one cannot control the weather (temperature, wind, rain) or species' emergences, within study study site control station(s) should be established, and on the ground pesticide levels should be measured in order to minimize controllable sources of variation.

The 22,000+ organisms that were identified during this survey represent more than 200 man-hours (10 hours per sample) of sorting, counting and identification, even though only the macrolepidoptera were counted to species. Identification of additional taxa to family or species would obviously lengthen this process, while a reduction in the number of Orders identified and counted would reduce the processing time per sample.

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Figure 3

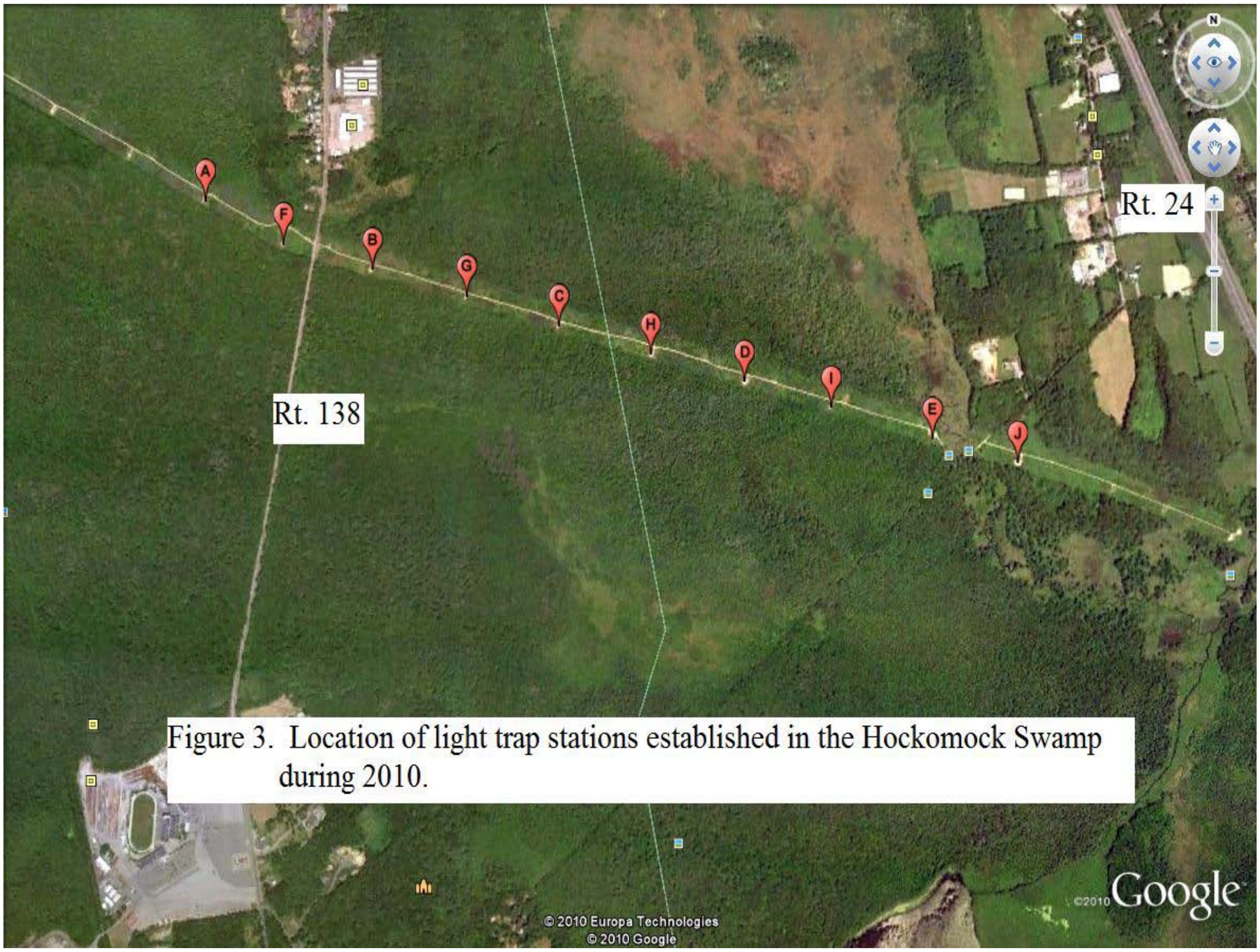

Figure 3. Location of light trap stations established in the Hockomock Swamp during 2010.

Figure 4

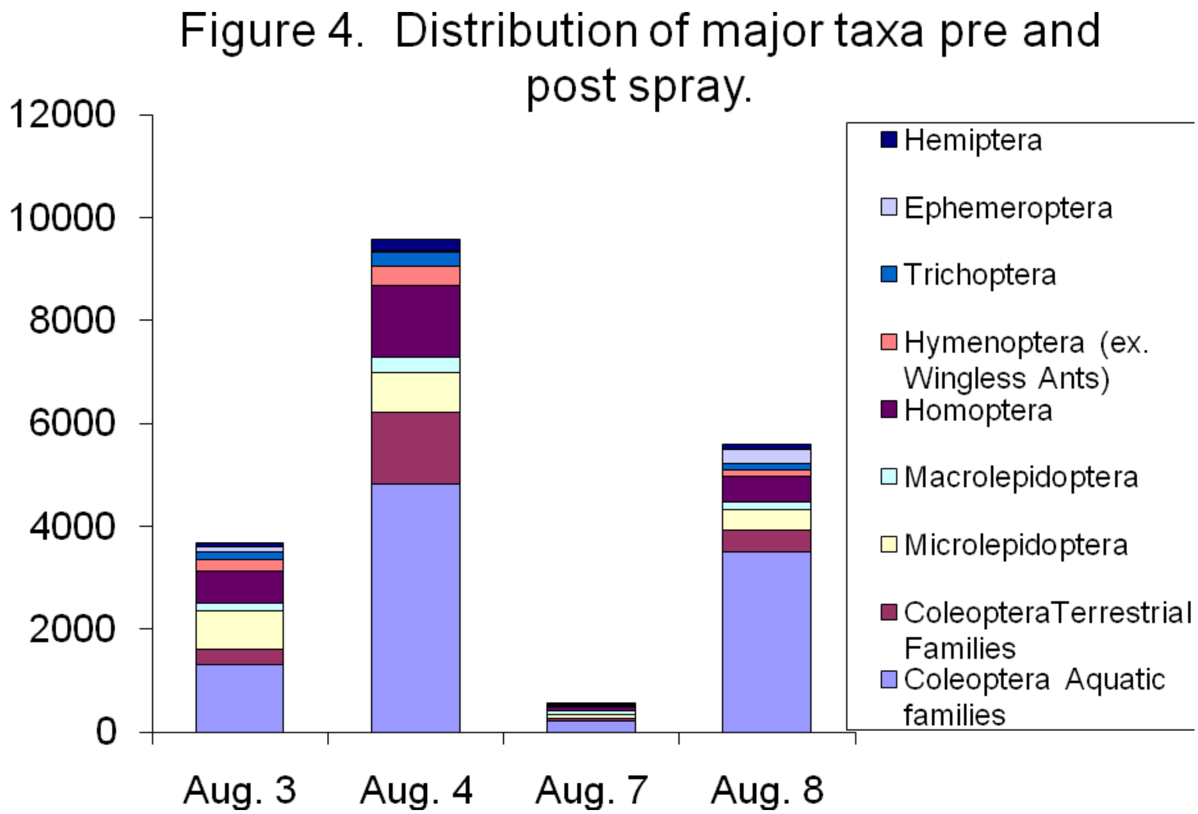

Table 1. GPS coordinates for the stations established for light trap sampling at Hockomock Swamp, August 2010.

Station code	N	W	
A	41.99909	-71.07156	
B	41.99728	-71.06525	
C	41.99577	-71.05819	Station block sampled on Aug. 3 and 7.
D	41.99427	-71.04787	
E	41.99274	-71.05116	
F	41.99796	-71.06862	
G	41.99656	-71.06168	
H	41.99501	-71.05471	Station block sampled on Aug. 4 and 8.
I	41.99358	-71.04787	
J	41.99210	-71.04080	

Table 2. Predominant vegetation located at the light trap stations surveyed at Hockomock Swamp during August 3-8, 2010.

D = dominanat | CD = co-dominant | P = presence greater than isc cover

Station	A	B	C	D	E	F
Vegetation						
Buckthorn	P		CD	CD	D	CD
Autumn olive	CD	P	CD	CD	P	CD
Red maple		P	P	CD	P	P
Gray birch	P	CD	P	CD	P	
Little bluestem & upl. Grasses	P	CD	P	P		P
Highbush blueberry		P	P	P	P	P
Goldenrod	P		P		P	P
Black cherry		P	P	P	P	P
Viburnum	P	P		P	P	
Phragmites	CD		CD	P		
Winterberry	P	P				P
Meadowsweet	P		P		P	
Sweet Fern		P		P		P
Red cedar	P			P	P	P
Black oak				P	P	
Wild indigo	P			P		
Pussy willow	P			P		P
Cattail		P		P		P
Grape	P		P			P
FW graminiods/sedges		P		P	P	
Tupelo			P			
Bayberry					P	
Winged sumac					P	
Silky dogwood					P	
Bush clover	P					P
Virginia creeper	P					
Steeplebush						
White piine						
Blackberry						
Aspen						
Poison sumac				P		
Ash			P			

Table 3. Summary of insects by Order, Family or other major grouping captured during the August 3-8 survey at Hockomock Swamp during 2010.
Boldface #'s = taxa subjected to statistial analyses.

			Pre-		Post	
	Max. Temp. °F.		86	90	84	87
	Min. Temp. °F.		63	72	54	57
	Date (August)		3	4	7	8
	Total By Order	Total by Family	Station block			
			(St.A-E)	(St. F-J)	(St.A-E)	(St. F-J)
COLEOPTERA	12059		1612	6246	248	3953
Terrestrial Families	**2155**		**296**	**1383**	**44**	**432**
Anthicidae		10	0	9	0	1
Anthribiidae		13	0	10	0	3
Bostrichidae		1	0	1	0	0
Cantharidae		37	2	20	2	13
Carabidae		1149	121	868	9	151
Chrysomelidae		14	0	12	0	2
Coccinellidae		348	85	214	8	41
Cucujidae		1	0	1	0	0
Curculionidae		17	4	10	0	3
Elateridae		5	1	1	0	3
Melandryidae		1	0	1	0	0
Mordellidae		2	1	1	0	0
Nitidulidae		2	0	2	0	0
Scarabaeidae		139	53	53	0	33
Silphidae		4	1	3	0	0
Staphylinidae		399	28	175	25	171
Tenebrionidae		13	0	2	0	11
Aquatic families	**9803**		**1296**	**4816**	**203**	**3488**
Dytiscidae		99	24	47	3	25
Haliplidae		60	8	40	1	11
Heteroceridae		66	8	28	22	8
Hydrochidae		422	12	153	29	228
Hydrophilidae		6763	484	3675	115	2489
Gyrinidae		3	0	2	1	0

Noteridae		70	16	19	0	35
Pselaphidae		54	7	36	0	11
Scirtidae		2266	737	816	32	681
Unident. Coleoptera		101	20	47	1	33
DIPTERA	**3357**		**1092**	**1431**	**222**	**612**
LEPIDOPTERA	2685		906	1070	159	550
Macrolepidoptera	**667**		**157**	**289**	**75**	**146**
Drepanidae		2	0	1	1	0
Geometridae		110	22	57	5	26
Lasiocampidae		1	0	0	1	0
Sphingidae		8	1	3	2	2
Notodontidae		12	4	5	1	2
Erebidae		344	71	153	47	73
Nolidae		2	1	1	0	0
Noctuidae		188	58	69	18	43
Microlepidoptera	**2018**		**749**	**781**	**84**	**404**
Nepticulidae		23	11	12	0	0
Cosmopterigidae		1	1	0	0	0
Oecophoridae		15	6	6	1	2
Tineidae		7	2	4	0	1
Lyonetiidae		8	3	4	1	0
Gracillariidae		27	12	11	1	3
Coleophoridae		493	185	186	30	92
Gelechiidae		426	198	128	18	82
Blastobasidae		10	2	7	0	1
Incurvariidae		10	9	0	1	0
Yponomeutidae		1	1	0	0	0
Plutellidae		1	0	0	0	1
Tortricidae		406	165	163	9	69
Cochylidae		9	0	4	2	3
Pyralidae		345	88	134	16	107
Crambidae		127	32	60	5	30
Pterophoridae		1	1	0	0	0
Unident. Microlepidoptera		108	33	62	0	13
HOMOPTERA	**2584**		**619**	**1410**	**68**	**487**
Aphididae		4	0	2	2	0
HYMENOPTERA	778					
Hymenoptera (ex. Wingless Ants)	**745**		**237**	**376**	**10**	**122**
Formicidae (wingless ants only)		33	10	12	1	10
TRICHOPTERA	**548**		**137**	**260**	**25**	**126**

EPHEMEROPTERA	**434**		**89**	**36**	**20**	**289**
HEMIPTERA	378		77	214	6	81
PSOCOPTERA	71		29	23	6	13
ODONATA	12		3	8	0	1
ORTHOPTERA	7		1	6	0	0
NEUROPTERA	5		1	3	1	0
MEGALOPTERA	2		0	1	0	1
DERMAPTERA	1		0	1	0	0
Spider	14		7	5	0	2
Total	22939		4820	11104	768	6247

Table 4. ANOVA tables for major insect groups documented at Hockomock Swamp pre (Aug. 3 & 4) and post (Aug. 7 & 8) a

	Source of variation	Df	SS	MS	Fs
Coleoptera – Aquatic families (9,803 individuals)					
	Subgroups	3	2611224.55	870408.18	
	A (pre post treatments)	1	293062.05	293062.05	2.34
	B (station blocks)	1	2315401.25	2315401.25	18.5
	A X B interaction	1	2761.25	2761.25	0.02
	Within subgroups	16	2002870	125179.38	
	Total	19	4614094.55		

	Source of variation	Df	SS	MS	Fs
Coleoptera – Terrestrial families (2,155 individuals)					
	Subgroups	3	205571.75	68523.92	
	A (pre post treatments)	1	72360.45	72360.45	4.93
	B (station blocks)	1	108781.25	108781.25	7.41
	A X B interaction	1	24430.05	24430.05	1.66
	Within subgroups	16	234886	14680.38	
	Total	19	440457.75		

	Source of variation	Df	SS	MS	Fs
Diptera (3,357 individuals)					
	Subgroups	3	169338.15	56446.05	
	A (pre post treatments)	1	142636.05	142636.05	49.59
	B (station blocks)	1	26572.05	26572.05	9.24
	A X B interaction	1	130.05	130.05	0.05
	Within subgroups	16	46018.4	2876.15	
	Total	19	215356.55		

	Source of variation	Df	SS	MS	Fs
Microlepidoptera (2,018 individuals)					
	Subgroups	3	64630.6	21543.53	
	A (pre post treatments)	1	54288.2	54288.2	29.6

	B (station blocks)	1	6195.2	6195.2	3.38
	A X B interaction	1	4147.2	4147.2	2.26
	Within subgroups	16	29347.2	1834.2	
	Total	19	93977.8		

	for nine major insect groups.						
Chi2 analysis by station block	**A-E Aug 3**		**F-J Aug 4**		**A-E Aug 7**		**F-J Aug 8**
	pre-spray		pre-spray		post spray		post spray
Aquatic Coleoptera							
$\sum(O - E)^2/E$	120.88		1526.07		79.49		714.44
$\chi^2_{(.01; 3)}$	11.35		11.35		11.35		11.35
Terrestrial Coleoptera							
$\sum(O - E)^2/E$	78.76		804.3		24.58		87.83
$\chi^2_{(.01; 3)}$	11.35		11.35		11.35		11.35
Diptera							
$\sum(O - E)^2/E$	26.04		120.81		77.11		19.51
$\chi^2_{(.01; 3)}$	11.35		11.35		11.35		11.35
Microlepidoptera							
$\sum(O - E)^2/E$	42.7		104.96		25.49		75.69
$\chi^2_{(.01; 3)}$	11.35		11.35		11.35		11.35
Macrolepidoptera							
$\sum(O - E)^2/E$	3.22		18.04		6.67		8.61
$\chi^2_{(.01; 3)}$	11.35		11.35		11.35		11.35
Homoptera							
$\sum(O - E)^2/E$	117.57		393.1		28.89		86.73
$\chi^2_{(.01; 3)}$	11.35		11.35		11.35		11.35

Hymenoptera minus wingless ants								
$\sum(O - E)^2/E$	208.59		26.29		3		18.1	
□$^2_{(.01; 3)}$	11.35		11.35		11.35		11.35	
Trichoptera								
$\sum(O - E)^2/E$	8		54.35		23.6		21.67	
□$^2_{(.01; 3)}$	11.35		11.35		11.35		11.35	
Ephemeroptera								
$\sum(O - E)^2/E$	202.18		92.61		15.5		408.61	
□$^2_{(.01; 3)}$	11.35		11.35		11.35		11.35	