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Maine CDC arboviral surveillance

Maine CDC coordinates arboviral surveillance throughout the state including mosquito monitoring as well as pesticide resistance monitoring. Funding for these activities varies by year, so this document represents what surveillance will be completed based on the amount of funding available:

Amount available	Surveillance activities
$25,000	Mosquito surveillance in York and Cumberland counties using light traps and resting boxes (EEE, WNV surveillance)
$50,000	Surveillance listed above as well as: mosquito surveillance in the Mid Coast area using light traps and resting boxes (EEE, WNV surveillance)
$75,000	Surveillance listed above as well as: mosquito surveillance in Augusta, Bangor, and Lewiston/Auburn areas. Surveillance will now include light traps, resting boxes, and GAT traps (EEE, WNV, *Aedes* species)
$100,000	Surveillance listed above as well as: mosquito surveillance in 1-2 additional areas (Aroostook county, Downeast)
$150,000	Surveillance listed above as well as pesticide resistance monitoring in up to two species
$150,000 plus	• Add or expand trapping sites • Add mosquito species to pesticide resistance monitoring • Add additional pesticides to pesticide resistance monitoring • Add additional pathogen testing (Jamestown Canyon or other emerging pathogens)

Created 3/14/2019

Maine Medical Center
Research Institute

End of Season Report 2018
MMCRI - Vector-borne Disease Laboratory
Scarborough, ME
Compiled by Charles Lubelczyk, Elizabeth F. Henderson, and Margret Welch

Mosquito Surveillance

Background

Mosquito trapping by MMCRI and its contractors occurred in several counties of Maine. The focus of the 2018 surveys was to continue surveillance in those areas with historical activity of eastern equine encephalitis virus (EEEV) with refined use of resting boxes to facilitate field collections of *Culiseta melanura*, the enzootic vector of EEEV. In addition, we concluded our second year of urban mosquito surveys, looking for container breeding (exotic) *Aedes* mosquitoes. A side endeavor examined relevant mosquitoes for the presence of Jamestown Canyon virus, which was detected for a second year, following two cases reported in 2017.

This report summarizes work completed by just MMCRI and its contractors, but does include information mosquito surveys conducted by the Maine Department of Agriculture, Conservation, and Forestry.

Methods

Trapping: In addition to the use of CDC Miniature Light Traps (baited with CO_2), resting boxes (n = 270) were widely deployed in June and kept at surveillance locations through the surveillance season, being removed in November. Mosquito surveys began during the first week of July and continued through the last week of September. Sampling at sites occurred once per week, with light traps placed in late afternoon and picked up before 10am the following morning. In urban locations in Biddeford/Saco, Portland, Augusta, Lewiston/Auburn and Bangor, up to twelve Biogents Gravid *Aedes* Traps (BG-GATs) were placed.

New sites in 2018 were chosen, for the most part, because of a geographic information system designed to identify prospective habitats for resting sites of *Cs melanura*. Parameters examined for site selection included a coniferous canopy, proximity to wetlands, proximity to public access areas, and distance to roads. Once surveillance sites were established for the season, resting boxes were placed adjacent to forested wetlands and remained in place throughout the season.

In 2018, surveys were conducted in the following communities – Alfred, Biddeford, Lebanon, Saco, Sanford, Waterboro, Eliot, and Kittery (York County); Harpswell, Portland, South Portland, Standish, Yarmouth (Cumberland County); Hiram (Oxford County); Augusta (Kennebec County); Auburn, Lewiston (Androscoggin County); Arrowsic, Georgetown (Sagadahoc County); Dresden, Edgecomb, Wiscasset (Lincoln County); Bar Harbor, Mount Desert, Southwest Harbor (Hancock County); Dennysville, East Machias, Machias (Washington County); Argyle Twp, Bangor, Old Town (Penobscot County); Cross Lake, Fort Kent, Saint Agatha, and Wallagrass (Aroostook County). In total, 115 sites were used for surveys in 2018 (Appendix 1).

Identification: Female mosquitoes were either frozen at -20°C or cold-shocked before identification. Mosquitoes were identified on a cold surface using a binocular dissecting microscope and pooled by site, trapping date, and individual species. Staff relied on the recently published key by Andreadis et al. (2005) to identify specimens. Identification keys were supplemented by Darsie and Ward (2005) and Means (1979, 1987).

Based on arbovirus response plan guidelines (DHHS 2017), identified mosquito species pools were stored at –80°C and those intended for testing were shipped on dry ice to the Maine Health and Environmental Testing Laboratory (HETL). Mosquitoes were submitted for testing in pools of 1-50 mosquitoes of a single species from one trapping site. In 2018, species of concern (enzootic or bridge vectors of West Nile virus or eastern equine encephalitis) that were submitted for testing included:

a. Phase I - July 1 through August 15, 2018 or first Maine or New Hampshire EEE or WNV detection (dates pertain to date of collection):

i. *Cs melanura, Cs morsitans, Cx pipiens, Cx restuans,* and *Cx pipiens/restuans*: Only these species will be tested. Any pool size may be submitted for testing but pool size cannot exceed 50 mosquitoes. As soon as EEE or WNV is detected in Maine or New Hampshire, mosquito submissions will follow phase II.

ii. Other mosquito species: During the mosquito season, please discard (or hold internally if interested) any mosquitoes that are not *Cs melanura, Cx pipiens, Cx restuans*, or *Cx pipiens/restuans.* Other mosquito species may be tested on a case by case basis, as resources and time allow. As soon as EEE or WNV is detected in Maine, mosquito submissions will follow phase II.

b. Phase II – August 15 or first Maine or New Hampshire EEE or WNV detection through October 1, 2018 (dates pertain to date of collection):

i. *Ae cinereus, Ae vexans, Cq perturbans, Cs melanura, Cs morsitans, Cx pipiens, Cx restuans,* and *Cx pipiens/restuan, Cx salinarius, Oc candensis and Oc sollicitans*

As Jamestown Canyon virus (JCV) is historically linked with early season, mammalian-biting mosquitoes (such as 'snowpool' *Aedes*), many of these are not routinely submitted for testing at HETL (Andreadis et al. 2008). As a consequence, these mosquitoes were stored at -80°C, until opportunistic testing could occur in the winter of 2018-2019. Testing focused on 'black-legged' *Aedes* spp (such as *Ae/Oc provocans*) as well as *Ae canadensis, Ae cantator, Ae sollicitans*, and *Cq perturbans.* Mosquitoes were tested in pools of 1-50 individuals of one species collected from one trapping site per date. RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA), and RT-PCR was performed using a primer pair targeting the small genomic sequence. Following the detection JCV by RT-PCR, the sample was purified and sent for sequencing to the University of Maine sequencing facility for confirmation. Mosquitoes tested for JCV were collected from the following counties – York (Alfred, Lebanon, Sanford), Cumberland (Yarmouth), Sagadahoc (Georgetown, Arrowsic), Lincoln (Dresden, Edgecomb, Wiscasset), Penobscot (Bangor), and Washington (Dennysville).

Results & Discussion

Mosquito collections: In total, surveys this year collected 10,304 mosquitoes over the course of the season. Despite lesser effort (# of traps in total), the proportion of collected specimens was still highest from CO2/light traps (Fig 1), as seen at two longterm surveys sites in York County. Overall, in light traps, *Cq perturbans*, several species of *Aedes,* as well as *Cs melanura* were most abundant. *Cs melanura* was the most abundant species in resting boxes, followed by *Ae canadensis, Cx territans, An puntipennis* and *Cs morsitans* (Table 1).

Mosquitoes collected with the BG-GAT were predominantly *Ae japonicus*, with *Ae triseriatus* and *Cx pipiens/restuans* also found, though in lesser numbers (Fig. 2). No *Ae albopictus* or *Ae aegypti* were collected, despite 232 trap nights of collections (Table 1).

Mosquito Testing: Collections from MMCRI and affiliates resulted in 486 pools (totaling 3,339 individual mosquitoes) submitted to HETL (Table 2). These submissions included the vector species mentioned above but also included occasional mosquitoes such as the exotic species *Ae japonicus.* Of the mosquitoes submitted for testing, four pools tested positive for WNV from August and September, with two pools collected from Penobscot and York Counties each. Positive pools were identified as *Culex pipiens/restuans* complex (Penobscot and York County) and *Cs melanura* (York County).

A total of 188 pools consisting of 3,333 individual mosquitoes were tested for JCV by MMCRI (Fig. 3). A total of 22 species were tested, with the highest numbers coming from specimens of *Ae canadensis, Ae cantator,* and *Cq perturbans.* Of the mosquitoes tested, two pools tested positive for JCV both collected on 7/19/2018, from two sites in Lincoln County. The positive pools were identified as *Ae sollicitans* and *Uranotaenia sapphirina.*

In 2018, the planning phase for the field season saw us rely on a GIS model developed in conjunction with the Maine Dept of Agriculture Conservation and Forestry, along with pre-season site visits to determine placement that would optimize sampling for placement of resting boxes. Species composition in boxes was not dramatically different from previous years however, with *Cs melanura, An quadrimaculatus, An punctipennis*, and *Ae canadensis* dominating (Table 1). Despite these increased numbers, no positive mosquitoes were collected during resting box surveys, possibly indicative of the low positivity in mosquito populations and the low numbers of mosquitoes collected per resting box. 2018 was a third consecutive year of below average rainfall for Maine, with low EEEV activity notice regionally, as well in the state.

This regional 'drought' may also explain low numbers seen at some survey locations, such as sites in Presque Isle (Aroostook County) (Fig. 4). Here, at both the Aroostook Farm and Manany Road locations, very few mosquitoes, particularly the target species *Cs melanura*, were collected throughout the season. Other sites associated with Presque Isle sampling, such as the Washburn School or Campground Road, found no mosquitoes.

In York County, other survey locations, such as the Massabesic Experimental Forest (MEF - US Forest Service) in Alfred and Waterboro, and Long Swamp Road in Lebanon, ME, maintained higher numbers of *Cs melanura* in resting boxes (Fig. 5) and light traps (Fig. 6), although at levels lower than some previous years. The MEF has been a site with recorded high numbers of Cs melanura, and like long Swamp Road is a consistent site for EEEV activity in vector mosquitoes. The MEF, in particular, has abundant breeding sites ('crypts') in the dominant red maple swamps, common throughout the forest (Dibble et al. 2007).

The contents of CO_2/light trap surveys on the MEF was from one site, Ida Jim Road, found that *Cq perturbans* remained the dominant species collected, followed by *Ae excrucians* (a nuisance mammalian biter) and *Ur sapphirina*. This site has been in use since 2010, with reliable collections of *Cs melanura*, but also relevant bridge vectors for EEEV including *Cq perturbans*, *Ae vexans*, and *Ae canadensis* (Fig. 6). In the past WNV-positive mosquitoes have also been recovered from the MEF.

Long Swamp Road has been used as a survey site since the 2009 EEEV epizootic (Gibney et al. 2011), and has also consistently produced similar vectors found on the MEF, with positive *Cs melanura* reported since 2009. Collections from light traps show that, although present, *Cq perturbans* was largely overshadowed by *Ae canadensis*, at least in 2018 (Fig. 7). But, with *Cs melanura* and *Ae vexans* still present, it remains an important site to monitor for EEEV activity. The final long-term monitoring site in York County, Shaw's Ridge Road, contains only a CO2/light trap, but data derived from this site was surprisingly sparse (Fig. 8). The site, on land owned by the town of Sanford, constitutes part of the Mousam River watershed. Town foresters severely thinned the forested habitat in spring of 2018. Increased light regimes and growth in adjacent wetlands by emergent vegetation post-silviculture, may have contributed to the reduced number of mosquitoes collected.

This year also marked the second year of surveillance on Mount Desert Island, working in cooperation with Acadia National Park (ANP) and the Somes-Meynell Wildlife Sanctuary. Because of a personnel issue, students from College of the Atlantic were not available for this year's survey, resulting in late sampling. As a consequence, we obtained relied on information from both resting boxes and a limited number of BG-GAT. The dominant species collected in resting boxes across several sites on MDI (private and federal land) were *Cs melanura* and *An punctipennis*, with Culex pipiens/restuans complex also found (Fig. 9). Of the sites sampled, Duck Brook Road (ANP) was exclusively *Cs melanura*, indicating that the GIS model used to predict optimal sampling locations worked correctly.

Although our overall mosquito activity in Maine was lower, on average, than regional estimates, WNV activity was present in the state, shown by collections of WNV-positive mosquitoes in both York and Penobscot Counties. All positive mosquitoes were collected later in the survey season (late-August-September), which is typical for the appearance of the virus. No mosquitoes tested positive for EEEV in 2018. Although Maine reported an additional case of Jamestown Canyon virus in September 2018, confirmation arrived later in the field season, after closeout of the surveillance program.

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Tables

Table 1. Statewide mosquito surveillance, Maine, 2018. Trapnights were – GAT: 232, Light/CO_2: 227, and resting box: 205.

species	GAT		Light CO2		Resting Box	
	count	number per trap night	count	number per trap night	count	number per trap night
Ae abserratus	0	0	23	0.10	0	0
Ae atropalpus	0	0	0	0	0	0
Ae canadensis	0	0	230	1.01	0	0
Ae cantator	0	0	1146	5.03	0	0
Ae cinereus	0	0	148	0.65	0	0
Ae communis	0	0	25	0.11	0	0
Ae decticus	0	0	0	0	0	0
Ae diantaeus	0	0	0	0	0	0
Ae dorsalis	0	0	0	0	0	0
Ae excrucians	0	0	95	0.42	4	0
Ae fitchii	0	0	27	0.12	0	0
Ae hendersoni	0	0	37	0.16	0	0
Ae implicatus	0	0	0	0	0	0
Ae intrudens	0	0	36	0.16	0	0
Ae japonicus	373	1.60	205	0.90	0	0
Ae provocans	2	0.01	105	0.46	0	0
Ae punctor	0	0	27	0.12	3	0.01
Ae sollicitans	0	0	169	0.74	0	0
Ae sticticus	0	0	8	0.04	0	0
Ae stimulans	0	0	61	0.27	1	0
Ae taeniorhynchus	0	0	2	0.01	0	0
Ae triseriatus	36	0.15	194	0.85	3	0.01
Ae trivittatus	0	0	14	0.06	0	0
Ae vexans	0	0	415	1.82	5	0.02
An barberi	0	0	11	0.05	3	0.01
An crucians	0	0	0	0	0	0
An earlei	0	0	0	0	0	0
An punctipennis	0	0	334	1.46	149	0.73
An quadrimaculatus	0	0	173	0.76	20	0.10
An walkeri	0	0	27	0.12	4	0.02
Cq perturbans	0	0	3588	15.74	19	0.09
Cs impatiens	0	0	0	0	0	0
Cs inornata	0	0	0	0	0	0
Cs melanura	2	0.01	47	0.21	300	1.46
Cs minnesota	0	0	0	0	0	0
Cx morsitans	0	0	2	0.01	29	0.14
Cx pipiens	0	0	0	0	0	0
Cx pipiens restuans	52	0.22	310	1.36	107	0.52
Cx restuans	0	0	0	0	0	0

Cx salinarius	0	0	17	0.07	0	0
Cx species	0	0	0	0	0	0
Cx territans	0	0	0	0	7	0
Ps ciliata	0	0	0	0	0	0
Ps columbiae	0	0	0	0	0	0
Ps ferox	0	0	1	0	0	0
Ur sapphirina	0	0	161	0.71	6	0.03
Wy smithii	0	0	0	0	0	0

Table 2. Vector-borne Disease Lab (VBDL) mosquito testing effort, 2000-2012.

Mosquitoes were shipped to the Maine Health and Environmental Testing Lab for testing by PCR.
Testing information includes a collaborator who ships pools to HETL through the VBDL (SWAMP Inc).

source	year	pools shipped	mosquitoes tested
Vector-borne Disease Lab	2001	156	918
	2002	380	2815
	2003	44	181
	2004	224	4230
	2005	128	831
	2006	319	2958
	2007	541	5153
	2008	539	5906
	2009	318	3182
	2010	382	2736
	2011	529	3385
	2012	907	16650
	2013	222	1127
	2014	255	2065
	2015	357	1810
	2016	330	1351
	2017	651	4317
	2018	484	3399
	Totals	6766	63014

Figures

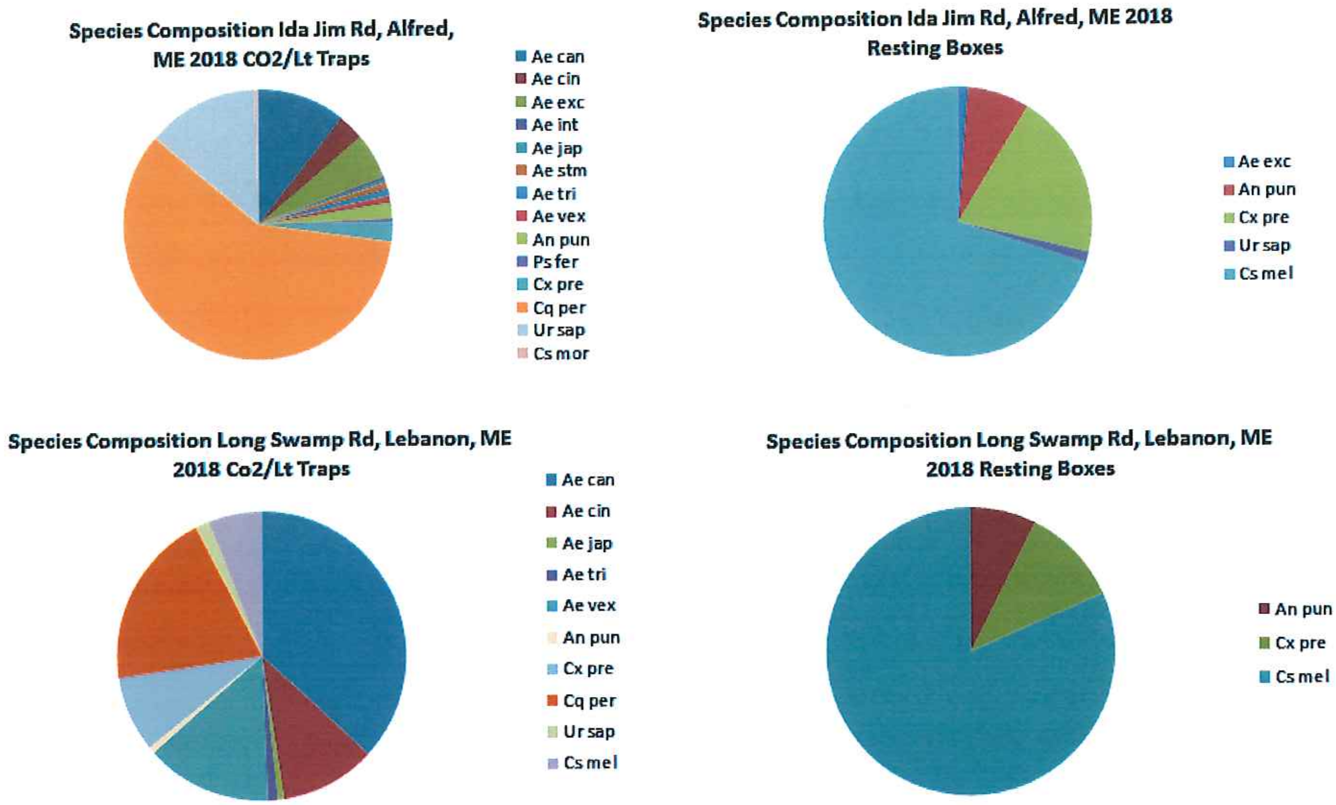


Figure 1. Composition of mosquitoes collected at two York County survey sites, CO_2/light traps vs resting boxes, July-September 2018.

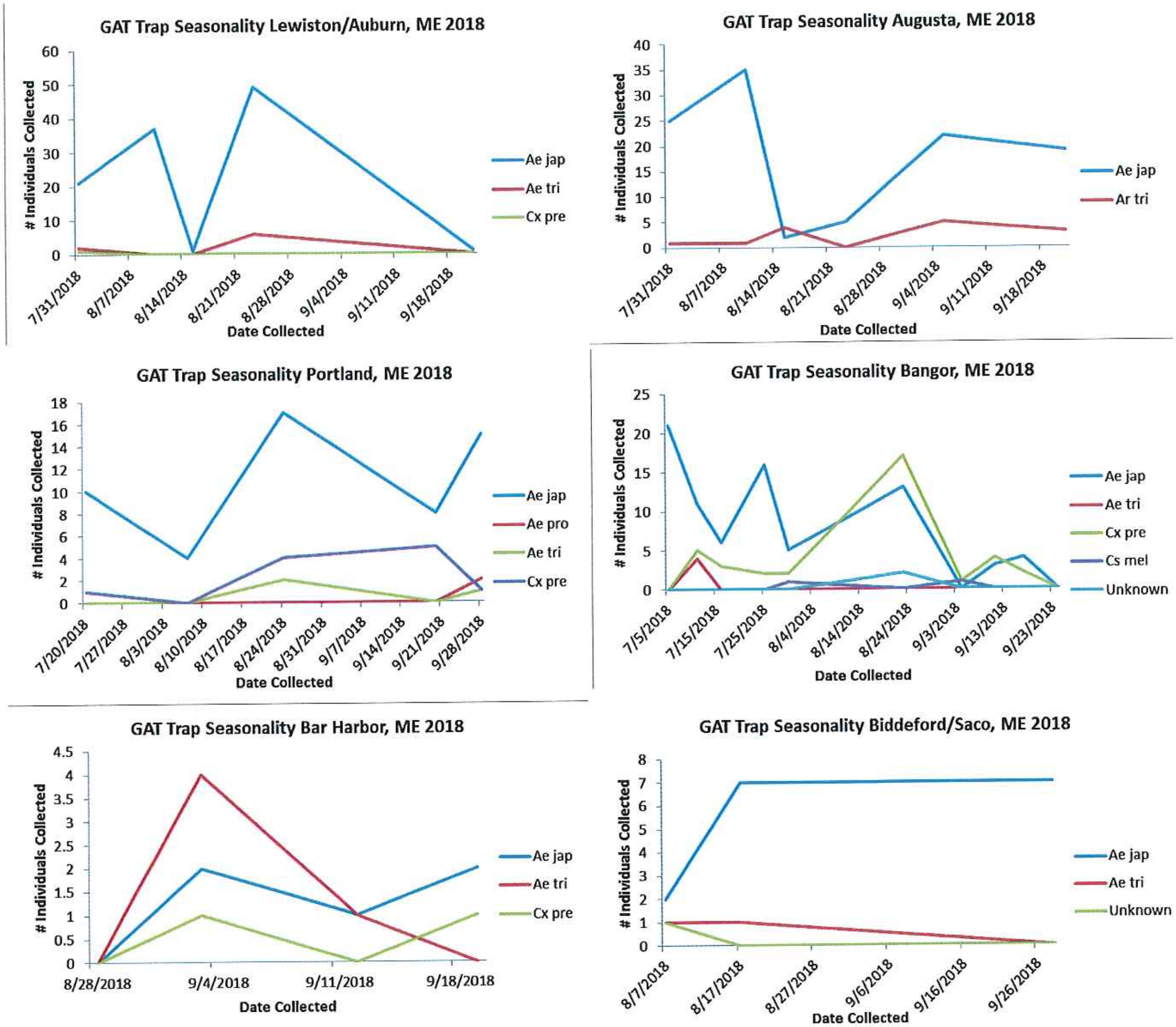


Figure 2. Species'seasonality of mosquitoes collected in BG GAT, July-Sept 2018.

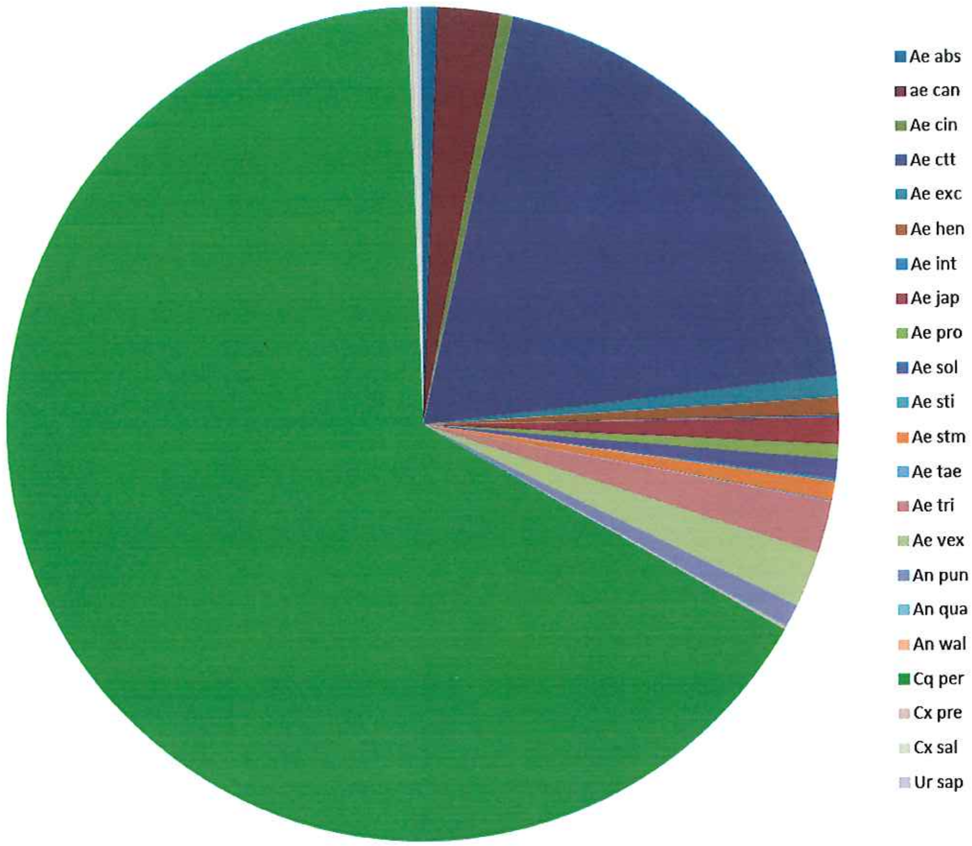
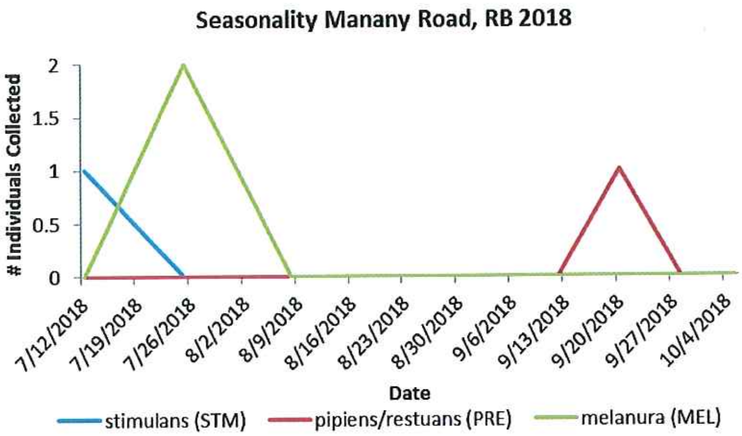


Figure 3. Species composition of mosquitoes tested for Jamestown Canyon virus, 2018.



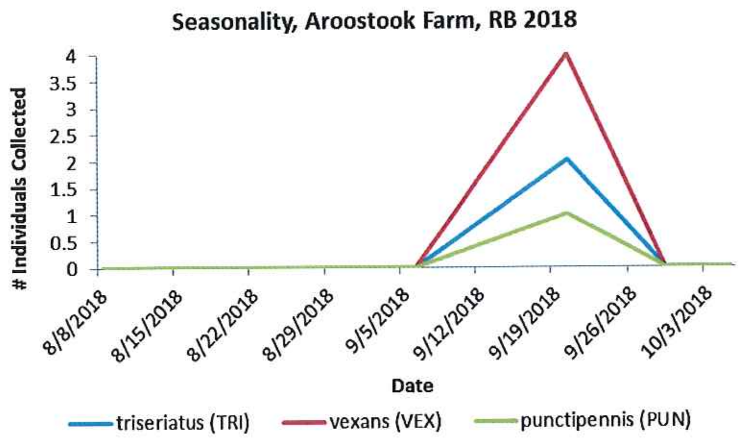


Figure 4. Mosquito collections from resting boxes at two traps sites, Presque Isle, ME (Aroostook County). July-October 2018.

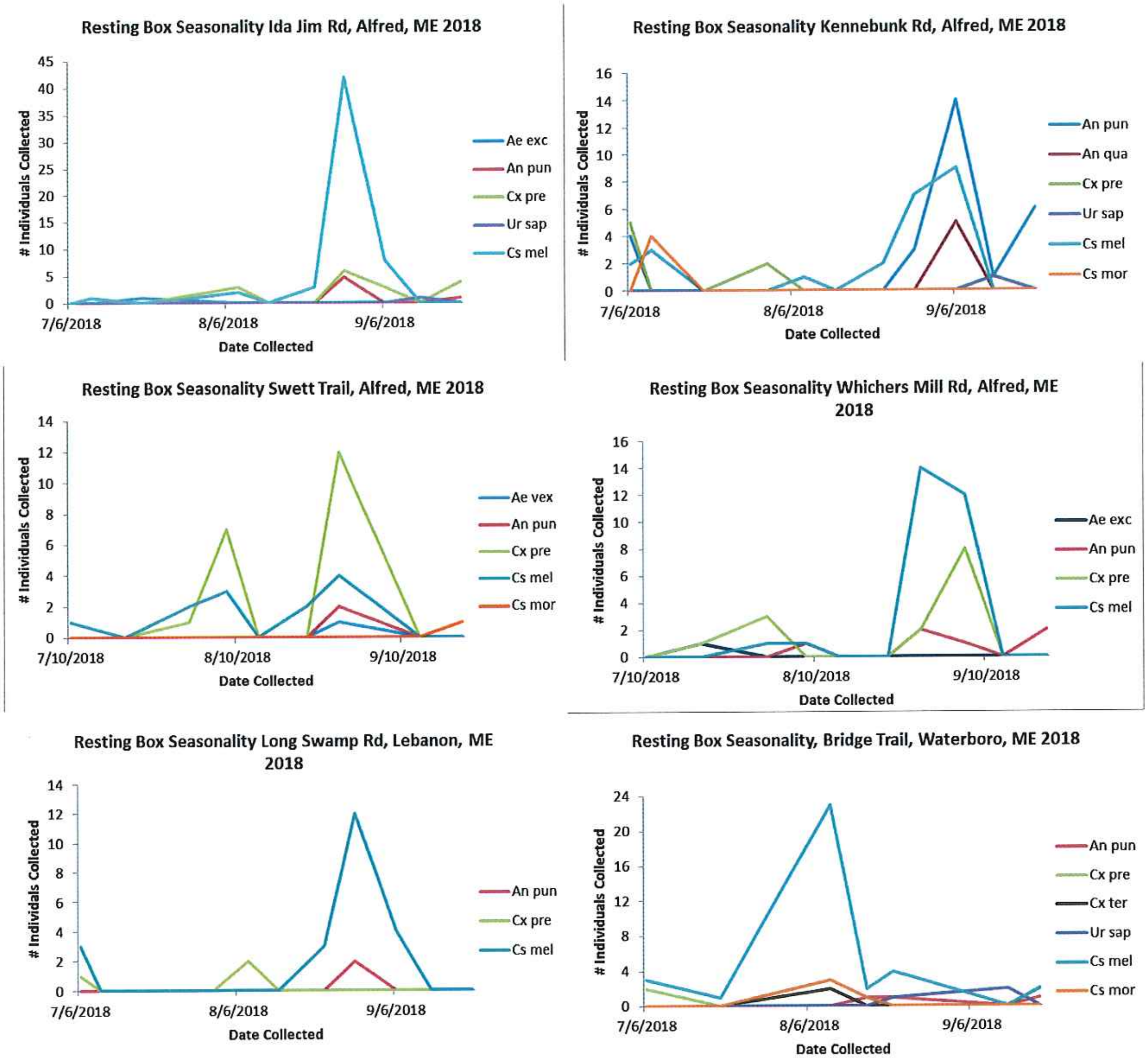


Figure 5. Seasonality of collections from resting boxes on the Massabesic Experimental Forest (York County). July-Sept 2018.

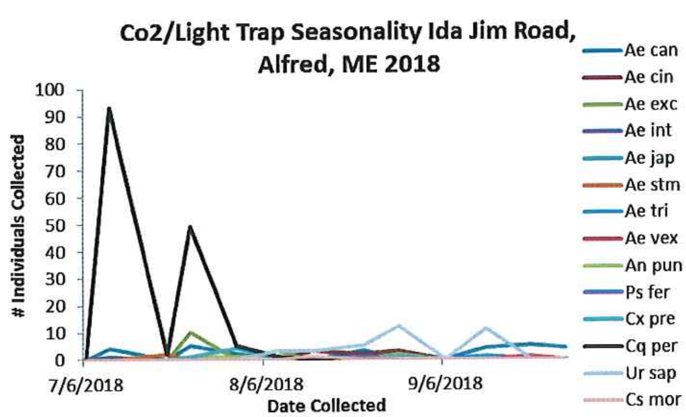


Figure 6. Seasonality of collections from CO2/light traps, Ida Jim Road (MEF). July-Sept 2018.

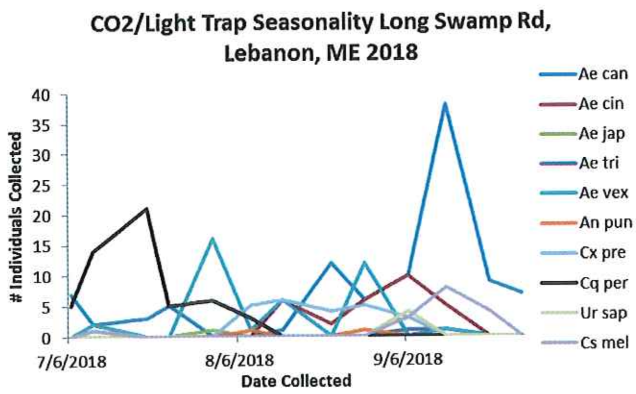


Figure 7. Seasonality of collections from CO2/light traps, Long Swamp Road (Lebanon, ME). July-Sept 2018.

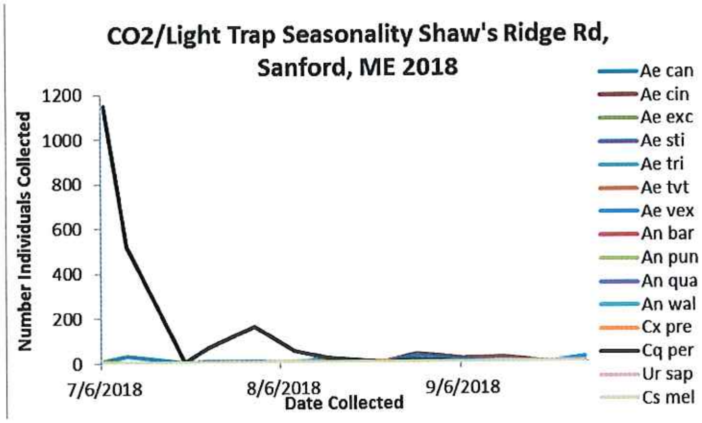


Figure 8. Seasonality of collections from CO2/light traps, Shaw's ridge Road (Sanford, ME). July-Sept 2018.

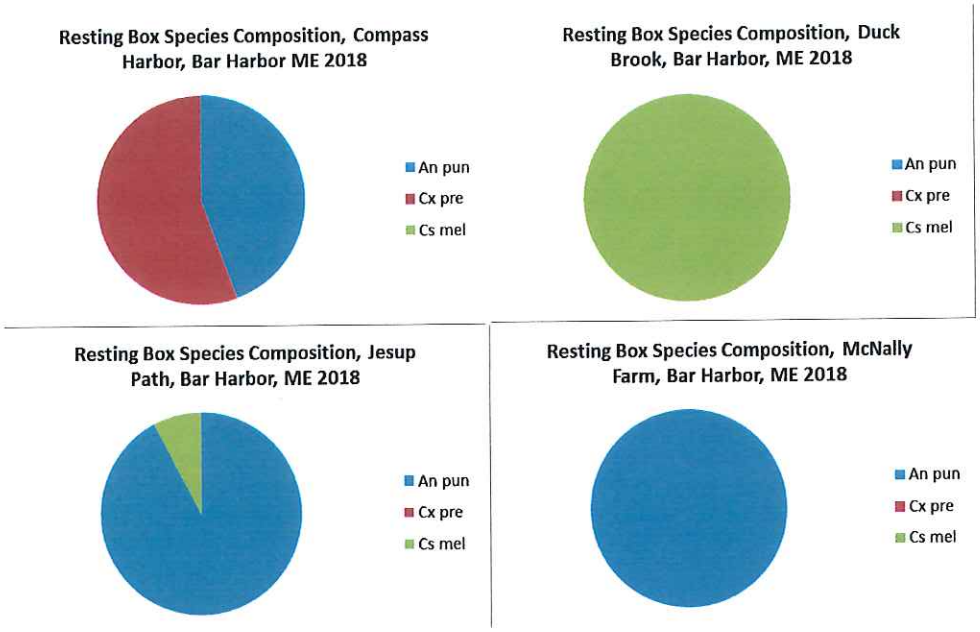


Figure 9. Mosquito composition of resting boxes from Mount Desert Island, ME. Aug-Sept, 2018.

Appendix 1. Survey sites, 2018

Appendix 1. Mosquito trpping locations, 2018.

Site Name	Lat	Long	Town	County	Trap Type	Notes
100 7th Street	44.79732	-68.78643	Bangor	Penobscot	CDC Gravid	
111 4th Street	44.79781	-68.7811	Bangor	Penobscot	GAT	
120 Leighton Street	44.81414	-68.77634	Bangor	Penobscot	Light	
124 Webster Street	44.79636	-68.79308	Bangor	Penobscot	CDC Gravid	
135 Forest Ave	44.80984	-68.76389	Bangor	Penobscot	GAT	
139 14th Street	44.80622	-68.79198	Bangor	Penobscot	GAT	
14 Coombs Street	44.80866	-68.76296	Bangor	Penobscot	CDC Gravid	
145 Allen Street	44.80276	-68.79787	Bangor	Penobscot	GAT	
149 Fountain Street	44.81501	-68.77695	Bangor	Penobscot	CDC Gravid	
15 Williams Street	44.79972	-68.78349	Bangor	Penobscot	Light	
15 Wood Street	44.80419	-68.79462	Bangor	Penobscot	CDC Gravid	
156 7th Street	44.79568	-68.78812	Bangor	Penobscot	Light	
19 Frances Street	44.80682	-68.79047	Bangor	Penobscot	Light	
194 Elm Street	44.81312	-68.76623	Bangor	Penobscot	Light	
199 Forest Ave	44.81203	-68.76444	Bangor	Penobscot	CDC Gravid	
204 Palm Street	44.81447	-68.76457	Bangor	Penobscot	CDC Gravid	
21 Poplar Street	44.81745	-68.77523	Bangor	Penobscot	Light	
26 Coombs	44.80875	-68.76227	Bangor	Penobscot	Light	
243 Grove Street	44.81276	-68.76668	Bangor	Penobscot	GAT	
306 Lincoln Ave	44.79511	-68.78864	Bangor	Penobscot	GAT	
31 Parkview Ave	44.80674	-68.76108	Bangor	Penobscot	CDC Gravid	
38 Forest Ave	44.80621	-68.76345	Bangor	Penobscot	GAT	
389 Pearl Street	44.8191	-68.75934	Bangor	Penobscot	CDC Gravid	
43 Manners Ave	44.80565	-68.79062	Bangor	Penobscot	CDC Gravid	
438 Birch Street	44.81953	-68.76175	Bangor	Penobscot	Light	
51 Poplar Street	44.81726	-68.77661	Bangor	Penobscot	Light	
67 Oak Point Lane	45.0945	-68.6533	Bangor	Penobscot	Resting Box	
70 Juniper Street	44.8172	-68.75706	Bangor	Penobscot	GATS	
73 Harthorn Ave	44.79635	-68.78897	Bangor	Penobscot	Light	
88 Poplar Street	44.81668	-68.77871	Bangor	Penobscot	GAT	
89 Cottage Street	44.80662	-68.78583	Bangor	Penobscot	GAT	
Babson Creek	44.373183°	-68.331119°	Mount Desert	Hancock	GAT	
Ball Field	44.327142°	-69.780786°	Augusta	Kennebec	GAT	
Boat Launch- Biddeford	43.473825°	-70.410131°	Biddeford	York	GAT	
Bridge Trail	43.550243°	-70.655775°	Waterboro	York	Resting box	
Broadway Gardens	43.628856°	-70.301214°	South Portland	Cumberland	GAT	
CAT	43.661233°	-70.245161°	Portland	Cumberland	GAT	
CC Road	43.568700°	-70.641956°	Waterboro	York	Resting box	
Cobscook Bay State Park	44.840258°	-67.148972°	Dennysville	Washington	CO2/Li	
Cole Road	43.476947°	-70.483531°	Biddeford	York	GAT	
Compass Harbor	44.373836°	-68.197469°	Bar Harbor	Hancock	Resting box/GAT	
Depot Road	43.894233°	-70.266908°	Standish	Cumberland	Resting box	
Depot Road B	43.893739°	-70.267864°	Standish	Cumberland	Resting box	
DHHS	44.099903°	-70.217553°	Lewiston	Androscoggin	GAT	
Dog Park	44.325528°	-69.771711°	Augusta	Kennebec	GAT	
Dresden Bog	44.104456°	-69.679592°	Dresden	Lincoln	CO2/Li	

Appendix 1. Survey sites, 2018

Site Name	Lat	Long	Town	County	Trap Type	Notes
Duck Brook Road	44.392989°	-68.230700°	Bar Harbor	Hancock	GAT/Resting Box	
Eastern Prom	43.669856°	-70.244275°	Portland	Cumberland	GAT	
Elm Street School	44.742044°	-67.390111°	East Machias	Washington	Resting box	
Flying Point Road	43.863258°	-69.754336°	Georgetown	Sagadahoc	CO2/Li	
Forest Grove Cemetery	44.315608°	-69.788774°	Augusta	Kennebec	GAT	
Gardner Lake Boat Landing	44.794292°	-67.382339°	East Machias	Washington	Resting box	
Gardner Lake- Lakeside West	44.753125°	-67.353636°	East Machias	Washington	Resting box	
Glassworks	44.101733°	-70.219661°	Lewiston	Androscoggin	GAT	
Hamilton Marine	43.661094°	-70.247561°	Portland	Cumberland	GAT	
Hannaford- Augusta	44.314700°	-69.764736°	Augusta	Kennebec	GAT	
Heath Road	43.426519°	-70.899444°	Lebanon	York	Resting box	
Heritage Trail	47.247297°	-68.574814°	Fort Kent	Aroostook	CO2/Li	
Holt Forest	43.869611°	-69.767183°	Arrowsic	Sagadahoc	CO2/Li	
Ida Jim Road	43.447556°	-70.681522°	Alfred	York	Resting Box, CO2/Lt	
Jellerson Road	43.555792°	-70.646642°	Waterboro	York	Resting box	
Jesup Path	44.419997°	-68.324567°	Bar Harbor	Hancock	Resting box/GAT	
Kennebec Humane Society	44.312831°	-69.800044°	Augusta	Kennebec	GAT	
Kennebunk Road	43.463125°	-70.688511°	Alfred	York	Resting box	
Landry Road	44.121014°	-70.205264°	Lewiston	Androscoggin	GAT	
Larabee's Landing	43.787769°	-70.161444°	Yarmouth	Cumberland	CO2/Li	
Laurel Hill Cemetery	43.492469°	-70.432903°	Saco	York	GAT	
Long Swamp Road	43.362914°	-70.872586°	Lebanon	York	Resting box, CO2/Lt	
Loring Memorial Park	43.672725°	-70.255122°	Portland	Cumberland	GAT	
Lubec Park	44.856376°	-66.988496°	Machias	Washington	Resting box	
McNally Farm	44.366244°	-68.210761°	Bar Harbor	Hancock	Resting box	
Mechanics Park	43.492403°	-70.448842°	Biddeford	York	GAT	
Mount Cutler	43.877131°	-70.805667°	Hiram	Oxford	GAT	
Mount Vernon Cemetery (Airport)	44.318844°	-69.788074°	Augusta	Kennebec	GAT	
Northeast Creek	44.425319°	-68.304419°	Bar Harbor	Hancock	GAT	
Outback Trail- UMaine Machias	44.707394°	-67.455028°	Machias	Washington	Resting box	
Perch Pond	44.9654	-68.7594	Old Town	Penobscot	Resting box	
Portland Trails	43.671231°	-70.249944°	Portland	Cumberland	GAT	
Reo Marina	43.644100°	-70.240828°	South Portland	Cumberland	CDC Gravid	
Ripples Pond	44.354928°	-68.351344°	Mount Desert	Hancock	GAT	
Riverside Cemetery A	44.109181°	-70.215500°	Lewiston	Androscoggin	GAT	
Riverside Cemetery B	44.108964°	-70.214139°	Lewiston	Androscoggin	GAT	
Saco School District	43.499856°	-70.438478°	Saco	York	GAT	
Saint Augustine	44.337925°	-69.783036°	Augusta	Kennebec	GAT	
Saint Mary's	44.103292°	-70.199461°	Lewiston	Androscoggin	GAT	
Savage Park	44.334431°	-69.760919°	Augusta	Kennebec	GAT	
Schmid Preserve	43.966869°	-69.613694°	Edgecomb	Lincoln	CO2/Li	
SFWMA	43.830411°	-70.606967°	Standish	Cumberland	Resting box	
Shaw's Ridge Road	43.458981°	-70.776656°	Sanford	Cumberland	CO2/Li	
Skate Park	44.090881°	-70.229589°	Auburn	Androscoggin	GAT	
SMCC	43.648539°	-70.228953°	South Portland	Cumberland	Gravid	Rapid response possible WNv case
Snowmobile Trail Above Fish River	47.205106°	-68.586483°	Fort Kent	Aroostook	CO2/Li	
Soldier Pond Brook	47.155581°	-68.579411°	Wallagrass	Aroostook	CO2/Li	
Somes Meynell Wildlife Sanctuary	44.357378°	-68.347081°	Southwest Harbor	Hancock	Resting box	

Appendix 1. Survey sites, 2018

Site Name	Lat	Long	Town	County	Trap Type	Notes
Sortwell Forest	44.013586°	-69.675586°	Wiscasset	Lincoln	CO2/Li	
1433 South Gate	45.0717	-68.6682	Argyle Twp	Penobscot	Resting box	
Spring Street	44.317125°	-69.781536°	Augusta	Kennebec	GAT	
Strawberry Lane	44.117817°	-70.208233°	Lewiston	Androscoggin	GAT	
Swett Trail	43.428869°	-70.668742°	Waterboro	York	Resting box	
UMaine Augusta	44.337536°	-69.797622°	Augusta	Kennebec	GAT	
UNE- Maintenance	43.455119°	-70.389622°	Biddeford	York	GAT	
UNE Water Treatment	43.459117°	-70.389191°	Biddeford	York	GAT	
USCG Station	43.643886°	-70.247178°	South Portland	Cumberland	CDC Gravid	Rapid response possible WNv case
USM- Lewiston	44.075486°	-70.171850°	Lewiston	Androscoggin	GAT	
West End Cemetery	43.646159°	-70.275695°	Portland	Cumberland	GAT	
Whichers Mill Road	43.436406°	-70.680100°	Alfred	York	Resting box	
Cundy's Harbor Road	43.800619°	-69.893847°	Harpswell	Cumberland	CO2/Li	
Job Road	43.740156°	-70.579950°	Standish	Cumberland	Resting box	
Libby Preserve	43.442925°	-70.730562°	Sanford	York	Co2/Li	Rapid response to positive horse
Rand Road A	43.801375°	-69.891428°	Harpswell	Cumberland	CO2/Li	
Rand Road B	43.802383°	-69.889825°	Harpswell	Cumberland	CO2/Li	
Thorncrag Bird Sanctuary	44.106822°	-70.178658°	Lewiston	Androscoggin	GAT	
Wallagrass Stream	47.167211°	-68.645772°	Wallagrass	Aroostook	CO2/Li	
Cyr Road	47.119925°	-68.319519°	Cross Lake	Aroostook	Resting Box, CO2/Lt	
Sinclair Road	47.158936°	-68.297993°	Saint Agatha	Aroostook	CO2/Li	

Appendix 2.

Throughout the past year Maine Medical Center Research Institute (MMCRI) Vector-borne Disease Lab (VBDL) has been working to establish the capacity in the state of Maine to add pesticide-resistance monitoring to our annual mosquito surveillance efforts. The early months of this process involved research to identify best rearing and pesticide resistance testing methods. This was followed by procurement of necessary equipment and pesticides. Once our supplies arrived we were able to begin to establish the insectary within which reference strains of mosquitoes would be reared. During this time troubleshooting of pesticide resistance testing methods has taken place. We are still determining if it is possible to test formulation pesticides or if strictly technical grade pesticides will work with our testing methods however we have successfully built the capacity to maintain mosquitoes to be used for pesticide resistance monitoring in the state of Maine. Details of each stage are outlined in the following paragraphs. Attached as appendices are: our monthly rearing schedule, CDC bottle bioassay methods, and the Vosshall Lab mosquito rearing methods.

Prior to establishing an insectary for pesticide resistance monitoring it was necessary to identify testing and rearing methods to be used as 'standard operating procedures'. We realized early in this process that adult mosquitoes and larval mosquitoes could not be tested via the same pesticide resistance bioassay because larval mosquitoes are aquatic and adult mosquitoes are terrestrial. This observation led us to identify the CDC bottle bioassay as a viable method for testing adult mosquitoes for pesticide resistance. The CDC bottle bioassay requires bottles be coated with a diagnostic dose of pesticide (a dose that will kill susceptible mosquitoes in 30-60 min) and outlines how to obtain this information. The CDC bottle bioassay does have some drawbacks, namely the methods outlined for cleaning bottles after use wouldn't actually clean the bottle of pesticide and the methods outlined for coating bottles are over simplified and could result in uneven coating of bottles. We corrected these issues by implementing cleaning methods known to clean bottles of chemicals and limiting bottle use to one diagnostic does of one pesticide. We corrected the bottle coating issues by obtaining a hot dog roller that rolls continuously when on. This ensures the bottle never stops rolling and is evenly coated with pesticide dilutions.

The larval pesticide resistance monitoring protocol identified was the WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides. We have not begun troubleshooting and implementing this protocol yet but plan to in the coming months. The WHO does have its own bioassay that is commonly used to test for pesticide resistance. We opted not to use that protocol because it requires the use of papers pre-impregnated with pesticide to be bought from WHO and used in their kit. Unfortunately they don't offer papers pre-impregnated with our pesticides of interest. As such, we went with the CDC bottle bioassay for adult testing and the WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides for larval testing.

We based our rearing protocols off of the mosquito rearing experience of Dr. Rebecca Robich, staff scientist for VBDL, and the Vosshall Laboratory Mosquito Rearing Standard Operating Procedures which are published online by Leslie B. Vosshall PhD from Rockefeller University.

The testing and rearing protocols informed the procurement process. After necessary equipment was identified those manufacturers not in our purchasing software (Lawson) needed to be added the the purchasing system. This was a time consuming process but once it was done equipment and supplies were easily purchased. During the procurement phase of establishing the insectary we identified a location to house the insectary. There was some debate as to whether it would be possible to house the mosquitoes at MMCRI or if another location was preferable. Ultimately it was decided that the best place to house the insectary would be at the University of Southern Maine's (USM) Gorham Maine campus. Dr. Joseph Staples from USM's Environmental Science and Policy Department offered the use of his lab space in Bailey Hall room 114A. The arrangement is working well and we have been able to not only have space for the insectary but access to a separate classroom within which the pesticide testing can be done. This enables us to minimize accidental pesticide exposure to mosquitoes housed within the insectary.

With identified Percival incubators as viable habitats within which mosquitoes can live. Two of these incubators are housed in the insectary. We are currently rearing a colony of Culex pipiens, obtained from Ohio State University, as our susceptible reference strain. This strain (Buckeye strain) was first established by Dr. Rebecca Robich in 2003. Our plan is to use this strain as our 'susceptible strain' by which we calibrate the CDC bottle bioassay. We calibrate the test by performing diagnostic dose response testing as outlined in the CDC bottle bioassay protocol. This enables us to ensure that we are comparing test results from wild mosquitoes against test results from a susceptible strain. Currently, we do not have enough of the Buckeye strain to perform the diagnostic dose testing however, two of the three adulticides we have chosen to test have diagnostic dose values published by the CDC. Starting testing with these adulticides (permethrin and sumethirn) enables our Buckeye strain to fully establish prior to subjecting a subset of Buckeye strain mosquitoes to diagnostic dose testing. This is ideal because the diagnostic dose testing potentially requires hundreds of mosquitoes. Testing this colony before it has had the chance to reproduce for a few generations could kill too many mosquitoes or could reduce the amount of genetic variation available to the mosquitoes that are left to reproduce.

We identified three adulticides to use with the CDC bottle bioassay: sumethrin, permethrin, and bifenthrin. We worked with Justin Adams of Swamp Inc. to identify pesticides that are both legal for use in Maine and commonly used by pesticide applicators. We initially procured formulation pesticide for use in the CDC bottle bioassay. Formulation pesticides are those used in the field by pesticide applicators. Formulations contain a percentage of active ingredient (pesticide) and a percentage of other, undisclosed, ingredients. The formulations used for initial testing were: Astro (active ingredient: permethrin), Anvil 2+2 (active ingredient: sumethrin), and Crosscheck (active ingredient: bifenthrin). Astro and Anvil were diluted so that there were 30ug/mL of each active ingredient, per CDC published diagnostic dose values1 for Culex pipiens. Dilutions of Astro and Anvil were introduced into 250 mL bottles, per CDC bottle bioassay protocol, and allowed to dry in bottles rotating on the hot dog roller overnight. Unfortunately, the formulation pesticide did not dry inside the bottles. I did a trial run in one set of Astro (permethrin) bottles and found that the majority of mosquitoes stuck to the sides of the bottle, this did not happen in the control (ethanol) bottles. A second set of Astro bottles were prepared and allowed to dry for 9 days, as was the Anvil bottle set. The formulation pesticide within the bottles still did not dry. It is possible that this is caused by the 'other ingredients' that the active ingredients are suspended in. To determine if ingredients inside the formulation pesticide are responsible for the dilutions not drying, technical grade pesticides (98%-100% active ingredient) were ordered and will be used in subsequent tests.

Early in the planning process we had determined that 4 or 5 mosquito species would be tested for pesticide resistance. As the insectary became functional this number was pared down to 1 mosquito species, Culex pipiens. Culex pipiens was chose as an initial focal species because it is easy to maintain in colony and we could obtain a subset of the Buckeye strain for use as a reference strain. Each species tested for pesticide resistance must have the chosen bioassay 'dialed in' or calibrated for use against a strain of the same species that is known to be susceptible. The Buckeye strain has been in colony long enough that any pesticide resistance that may have been present in the colony founders has likely been bred out. This can be confirmed via sequencing methods designed to identify genetic mechanisms for pesticide resistance. We will need to identify susceptible strains to calibrate pesticide resistance bioassays against for each species we eventually test. Currently, we are focusing our efforts on Culex pipiens because both a reference strain and wild populations are available. Culex pipiens has also been identified as a potential bridge vector of West Nile Virus and, as such, is a species of interest to the vector-borne disease community.

Mosquitoes are maintained at 25°C in 70%-80% relative humidity with at 15 hour light, 9 hour dark light cycle. Each cage of adult mosquitoes are house in a clear trash bag. The trash bag acts as secondary containment and helps trap humidity inside the cage. Adult mosquitoes are offered damp sponges and 10% sucrose around the clock unless a testing or blood feeding protocol requires removal of sucrose the day prior to testing/blood feeding. Whole blood from chickens is used to feed our Buckeye strain. Blood feeding is accomplished via a glass membrane feeder and a parafilm membrane. Feeding mosquitoes on a membrane feeder eliminates the need to feed them on live animals. Blood is kept warm during feeding via water pump submerged in water warmed to 35°C. Gravid females are offered a container of water within which they can lay their eggs. Egg rafts are removed soon after laying and placed in individual larval pans. Newly hatched larvae are provided approximately 1/8th" of a rabbit food pellet and 1/16 tsp of finely ground tetramin tropical fish food. The same quantity of fish food is provided in larval pans daily.

Egg rafts from field sites are obtained by placing black restaurant bussing tubs at field sites and pouring about 1" of hay broth into the tub. Egg rafts are collected the next morning and returned to the lab. Each egg raft is placed in its own larval pan for ease of identification once the 4th instar stage is reached.

During the past year the VBDL has built the capacity to maintain both wild and colony strains in an insectary house on USM's Gorham campus. We have identified a species of mosquito that is stable in the insectary and can be used to troubleshoot rearing and testing methods and have successfully maintained this species in the insectary for a few months. We are in the process of identifying whether formulation pesticide, technical grade pesticide, or both are viable for use in our chosen pesticide resistance monitoring protocol. Our next steps will be to test technical grade pesticide in the CDC bottle bioassay and to develop diagnostic dose response values with our Buckeye strain.

References

1. https://www.cdc.gov/zika/vector/insecticide-resistance.html