Preliminary Faunistic Survey of Mosquito Species (Diptera: Culicidae) with a Focus on Population Densities and Potential Breeding Sites in Greater Portland, Maine

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Abstract

In the spring of 2001, a concerted effort was undertaken by the Maine Forest Service, Maine Medical Center, Maine Department of Human Services Bureau of Health and the University of Maine to gain a better understanding of mosquitoes populations in Maine in relation to their potential for transmitting West Nile Virus (WNV). This project was divided into four separate studies. For this part, the Maine Department of Conservation, Forest Service, Forest Health and Monitoring Division focused primarily on an intensive mosquito survey and monitoring program in Portland, Maine. This project involved the identification of mosquito species and the location and assessment of potential mosquito breeding sites.

Of the 39 mosquito species currently listed from Maine, a total of 27 adult species were collected from Portland in this study. Larvae of twelve species were also collected and breeding sites identified. **The first specimen of *Aedes japonicus* (Theobold), from Maine, was also collected on June 26, 2001 in Portland in a CDC gravid trap, bringing the list of mosquito species from Maine to 40 with a total of 28 adult species collected from the Portland study.** Adults of twelve of the Maine species are reportedly vectors of the West Nile Virus.

Acknowledgements

Thanks to Richard Dearborn, Maine Forest Service, for his guidance, expertise and support throughout this project and editing the final version of this report. I would also like to thank Charlene Donahue, Maine Forest Service, for photography and help in preparing a database; Jessica Barbay, 2001 summer intern for the Margaret Chase Smith Government Internship Program from the University of Maine, for assisting in trap setup, larval collections, and the pinning and labeling of mosquito specimens; Dr. John Burger, professor of Biology, University of New Hampshire, for the in-depth mosquito taxonomy courses he coordinated and instructed, his expertise and willingness to assist us in mosquito identification; Dr. Paul Reiter, CDC, for insight on 1st instar larval differences between *Culex pipiens* and *Culex restuans*; Geoff Beckett PA-C, MPH, Assistant State Epidemiologist, Maine Department of Human Services, Bureau of Health for allowing use of his property as a selected trapping site; Bradley Roland, Project Engineer, City of Portland Department of Public Works for GIS information and maps; Henry Dresch, PE from the Portland Public Schools for allowing access to school property around Portland and in handing out mosquito awareness pamphlets. I would also like to extend thanks to the Portland Wastewater Treatment Facility staff and Richard Brzozowski, Extension Educator, University of Maine Cumberland County Cooperative Extension office, and a number of Portland residents for allowing access to their property for this survey.

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Introduction

In the spring of 2001, an intensive mosquito survey program was initiated in Portland, Maine. Portland was chosen based on the high risk factor for vector related incidence because human population in this part of the state is large and is in close proximity to migratory bird flyways and where WNV has already been identified. This program was conducted to establish baseline faunistics of mosquito species, population densities and to locate and assess potential breeding sites in the Greater Portland area in conjunction with Centers for Disease Control (CDC) West Nile Virus (WNV) protocol. Along with identification of species, seasonal distributions and identification of larval habitats were also assessed. The results of this study will be used to assist in monitoring mosquito population densities in relation to human populations for future disease management strategies. A variety of techniques were used to assess mosquito populations. Light trapping, gravid trapping, egg raft sampling and larval dipping were used throughout the season.

Before the onset of this project, 39 species were listed as occurring in Maine (McDaniel). In addition to determining seasonal and spatial distribution of these species, there was special emphasis focused on whether *Aedes japonicus* (Theobald) and *Aedes taeniorhynchus* (Wiedermann), two new exotic species, had yet become established in Maine.

Mosquitoes (Diptera: Culicidae) are very common insects and are present throughout the world. Mosquitoes can transmit an array of disease organisms. There are four stages in a mosquito's life cycle; egg, larva (four instars), pupa and adult. Adult females bite to acquire the blood necessary for egg laying. The males do not bite. Different species of mosquitoes feed on different hosts. For example: *Culex territans* (Walker) feeds primarily on amphibians/reptiles while *Culiseta melanura* (Coquillett) prefers birds, and *Aedes canadensis* (Theobald) does not seem to have a preference and is known to feed on birds, mammals, and/or amphibians.

Revised Version

May 2002

When it became necessary to print more copies of this report the authors felt that additional data on larval populations and seasonality (pp 34- 36) should be added to further clarify population assessment issues. This information was from surveys conducted during the 2001 project season. In addition, we would like to point out that most literature now places all of our species of *Aedes* except two, *A. cinereus* and *A. vexans*, in the genus *Ochlerotatus*. We did not make this change in this report but urge our readers to keep this in mind when comparing reports.

Materials

6 CDC miniature CO_2 light traps
6 blue plastic ½ gal. insulated water containers with string and clips for dry ice
400 lbs dry ice (CO_2)
10 6V batteries for CDC traps
1 battery charger
1 large plastic cooler with lid
3 CDC gravid traps model 1712, JW Hock Co.
1 spare parts bag for traps
2 bags of large plastic petri dishes with covers
hay infusion (1 lb hay per 30 gal water, fermented for 1 week)
2 large 5 gal. plastic pickle buckets
3 plastic 7x12x10 tubs for gravid traps, painted black
6 black plastic 7x12x17 restaurant bus pans
18, 24 well microplates with covers
1 standard mouth aspirator*
1 battery operated insect aspirator
50/50 Isopropyl (rubbing) alcohol 70%/water
3 black 5 gal. buckets
1 aquatic pipette for larvae
larval forceps and microslide tool sets
4 packages of glass vials with screw caps
1 large plastic larval tray
1 small plastic larval tray
1 larval dipper with extendable handle
latex gloves
2 BioQuip mosquito breeding containers
1 pocket thermometer
cotton
1 GPS unit
eyedroppers
1 vial tray
glass micro vials
Cornell insect drawers
unit pinning trays
1 pinning block
#2 insect pins/ labels/ points/ shellac gel

* Although the standard mouth aspirator was used when collecting adult mosquitoes during this project, information suggests that the use of these types of aspirators should be discouraged when dealing with vector related species due to the risk of contracting disease. The battery operated insect aspirator is an appropriate alternative for projects such as these.

Methods

This project started in Portland, Maine on May 21, 2001 and ceased on August 14, 2001. There were six sites in Portland selected for CDC CO_2 light traps and egg raft sampling. These sites were located in East Deering, East End, West End, Oakdale, Rosemont and Riverton neighborhoods. Three of these sites were chosen for gravid trapping; East End, Oakdale, and Rosemont. The selection of trapping sites was based primarily on human population and security. There were also sixteen larval dipping sites monitored regularly in the Greater Portland area. The field work took place on Monday and Tuesday of every week for eleven weeks. Monday morning work consisted of picking up the dry ice, setting up traps and egg raft sampling pans at the chosen sites and larval dipping. Tuesday work consisted of collecting, counting and freezing mosquito specimens found in the traps, and collecting egg rafts from the pans. The traps were collected in the same order as they were set, allowing for approximately 24-hours of collection time. Date, time, air and water temperatures and current weather conditions were recorded on both days on a field data sheet (Appendix A). Traps were always cleaned to remove any insect parts and scales that might be left. The last three days of the week consisted of sorting, mounting, labeling and identifying the specimens collected. Processing specimens on a weekly basis ensured that the mosquitoes were in excellent condition and they were not backlogged.

Batteries were fully recharged and hay Infusions were prepared for fermentation on Monday nights for the following weeks trapping session.

Identifications were done using a binocular dissecting microscope, and standard keys (Wood Dang and Ellis, Darsie and Ward (1981), Burger, Carpenter and LaCasse).The specimens and pertinent information were recorded in a database. Adult and larval dip specimens have been deposited in the Maine Department of Conservation, Forest Service, Insect and Disease Lab insect collection in Augusta, Maine for future reference. Egg rafts were identified after hatching and species at 1st instar were recorded. These specimens were not retained in the collection.

Trapping

The CO_2 traps used were standard **CDC miniature light traps** which were hung from plastic insulated water containers filled with 5 lbs. of dry ice and then hung approximately 5 feet off of the ground (Photo 1). The CO_2 gas to escapes through a small hole in the bottom and then the escaping CO_2 gas flows down over the black plastic lid that shields the trap. The CDC miniature traps were attached to a battery that supplies power to the light and fan. The CO_2 gas and light attracted adult, biting mosquitoes, and then the fan sucked them into the collection container. These traps were placed at six sites throughout Portland. At five of the sites they were hung from tree limbs, at one site the trap was hung from a bracket placed on the side of a shed by the owner of the property. During collection of trap contents, the fan remains operable while securing individuals inside the collection container. The container was removed and placed in a cooler with dry ice. After the sample was frozen (5-8 minutes) the non-mosquitoes were discarded and the adult mosquitoes were counted, recorded (Appendix A.) and placed (using forceps) into dry petri dishes and placed carefully back into the cooler for transport. The individual adult mosquitoes must be handled as little as possible so as not to remove scales and other identifiable characters. The trap was dismantled, cleaned and removed from the site until the next collection week.

Photo 1. Assembled CO_2 light trap.

The gravid traps used were **CDC gravid traps**, model 1712, from JW Hock Co., with the interior of the tubs painted flat black (originally light blue). These traps were also battery operated to run a fan that sucked egg laying female mosquitoes into a collection net (Photo 2.). The tubs were filled with a hay infusion recommended by the CDC to about two inches inside the tub. The collection unit was placed over the tub so that the collecting tube rested about 1 ½ inches above the level of the infusion. During collection of trap contents, the fan remained operable while securing individuals inside the net. The net was removed and placed in a cooler with dry ice. After the sample was frozen (5-8 minutes) the non-mosquitoes were discarded and the adult mosquitoes were counted, recorded (Appendix A.) and placed (using forceps) into dry petri dishes and placed carefully back into the cooler for transport. The remainder of the trap was dismantled, cleaned and removed from the site until the next collection week. The tub was left at the site for larval sampling and recharged every Monday with about ½ inch of additional fresh infusion.

Photo 2. Operational gravid trap.

Egg Raft Sampling

Egg raft sampling at the six sites was initiated on July 2, 2001. The CDC recommended infusion of hay and water was added to a depth of three inches in a black restaurant bus pan, and set at the same time as the other traps (Photo 3). The pan was placed near or under vegetation in a shaded spot. The following day, the egg rafts were collected, counted, and placed individually into 24-well microplates, the wells were filled half-way with the same hay infusion. Date, time, air and water temperature, number of rafts and current weather conditions were recorded (Appendix A). Each microplate was then covered for transport. Microplates with egg rafts must be stored away from heat and direct sunlight, until hatching. The bus pans were then dumped and removed from the site.

Photo 3. Placing an eggraft pan at selection site.

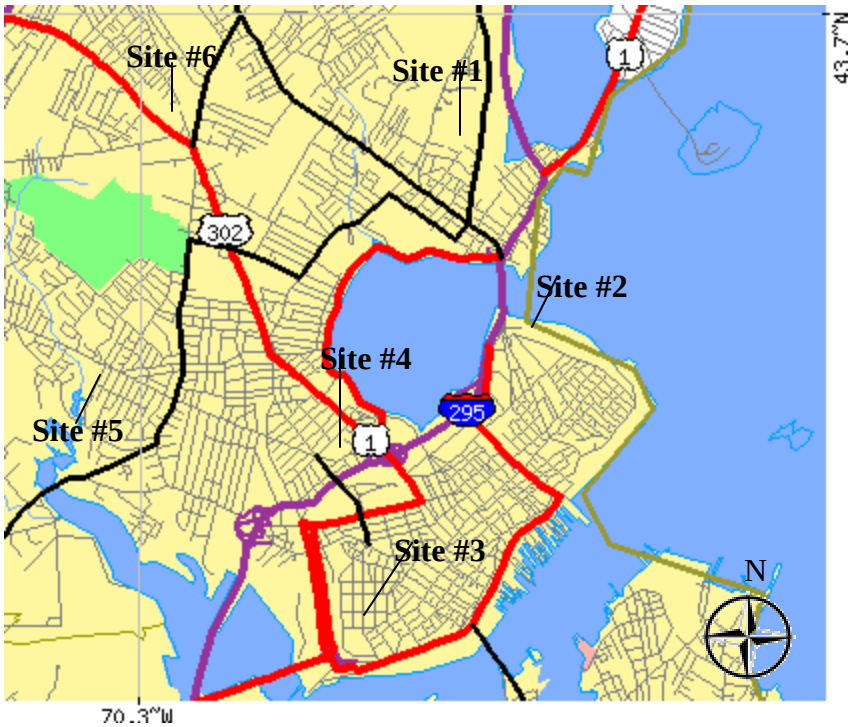
Larval Dipping

Larval dipping took place on both field days, every week. There were 16 sites monitored on a regular basis for larvae. Artificial containers as well as ponds, marshes and puddles were sampled using a standard mosquito larval dipper with extendable handle. Three scoops (350 ml each) of water were taken at each site (Photo 4). Larvae from those dips were extracted, using eyedroppers, and placed into glass vials with screw caps. The larvae were preserved in a 50/50 mix of water and 70% isopropyl alcohol. Date, time, air and water temperature, number of individuals and current weather conditions were recorded (Appendix A).

Photo 4. Larval dipping at Maggie Circle housing development construction site in the Riverton neighborhood.

Site Description

Portland, Maine - Greater Portland Area



Portland, Maine is located in the Portland West USGS topographic map quadrant N43°373, W70°150 in the coastal-southwestern part of the state of Maine. It is Maine's largest city, with a population in 2000 of 64,249 persons (US Census). Six sampling points, referenced by neighborhood, were selected within the Greater Portland area to represent different demographics. Neighborhoods selected were Deering, East End, Riverton, Oakdale, Rosemont, and West End. Population estimates were obtained using 2000 Census tract data. Each site contained one CDC light trap, and one bus pan for egg raft sampling. However, only three sites, Rosemont, East End and Oakdale, were chosen for gravid trapping. Sixteen larval sampling sites were located within these six neighborhoods. GPS coordinates and characteristics of each plot were noted.

Site #1-East Deering and Deering neighborhoods. Approximate human population: 7,691

Trapping site characteristics- CDC CO_2 trap and egg raft sampling pan. Empty lot on corners of Kidder and Grafton Streets. Dense hardwoods and vegetation with moist ground. Level terrain.

Larval sampling sites- Pond and temporary woodland melt water pools at Baxter Woods, a 30-acre nature preserve. Black bucket, with hay infusion, placed in wooded area. Washtub, at

a residence, located off Grafton Street. Puddle and flooded grassy area at Portland's Bayside Park off Baxter Boulevard. Urns and ponds at Evergreen Cemetery.

Site #2-East End neighborhood. Approximate human population: 10,107

Trapping site characteristics- CDC CO_2 trap, gravid trap and egg raft sampling pan placed at the Portland Wastewater Treatment Facility. Pines, tall grassy areas. Sloping terrain.

Larval sampling sites- Gravid tub, with hay infusion, placed at the Portland Wastewater Treatment Facility. Tires found on the side of the road on the Eastern Prom Walkway. Tires, empty plastic trashcan lid, black bucket used for roofing tar and a washtub in between buildings off of Lancaster Street.

Site #3-West End neighborhood. Approximate human population: 13,337

Trapping site characteristics- CDC CO_2 trap placed at a residents home on Bowdoin Street. Egg rafting pan placed in trees at Vaughn Street Cemetery.

Larval sampling sites- Black bucket with hay infusion placed near dumpster behind a deli on Brackett Street. Pond at Deering Oaks Park.

Site #4-Oakdale neighborhood. Approximate human population: 5,518

Trapping site characteristics- CDC CO_2 trap, gravid trap and egg raft sampling pan placed at the University of Maine Cooperative Extension building, 15 Chamberlain Avenue.

Larval sampling sites -Tires found at the Portland Water District office located on Douglass Street. Gravid tub, with hay infusion, placed at the University of Maine Cooperative Extension building on Chamberlain Avenue.

Site #5- Rosemont neighborhood. Approximate human population: 4,496

Trapping site characteristics- CDC CO_2 trap, gravid trap and egg raft sampling pan placed at a residence on Hastings Street.

Larval sampling sites- Capisic Pond, an 18 acre nature preserve and Portland's largest freshwater pond. A small freshwater marsh on the corners of Stevens Avenue and Congress Streets.

Site #6- Riverton neighborhood. Approximate human population: 13,127

Trapping site characteristics- CDC CO_2 trap placed in woody area off of Eleanor Street. Egg raft sampling pan placed at Maggie Circle housing development. Primarily hardwoods, dense canopy, very damp area.

Larval sampling sites- Small pond behind the DEP office on Canco Road. Temporary pools and permanent marsh areas created by housing construction in Maggie Circle Development.

Results and Discussion

All mosquito species discussed in this section were collected during the 2001 project season in greater Portland, Maine. Individual species seasonality, peaks of activity, anecdotal species information, egg raft sampling results, larval sampling results and notes for known mosquito WNV vector species (CDC 2001) and other species collected are presented along with site trapping totals and greater Portland, Maine trapping totals. These results are presented in condensed form for adult mosquitoes in Appendix B (page 33) and for mosquito larvae in Appendix C (page34). **A new species for Maine, *Aedes japonicus* (Theobald), was collected in Portland on June 26, 2001 at trapping site # 4 in a CDC gravid trap.**

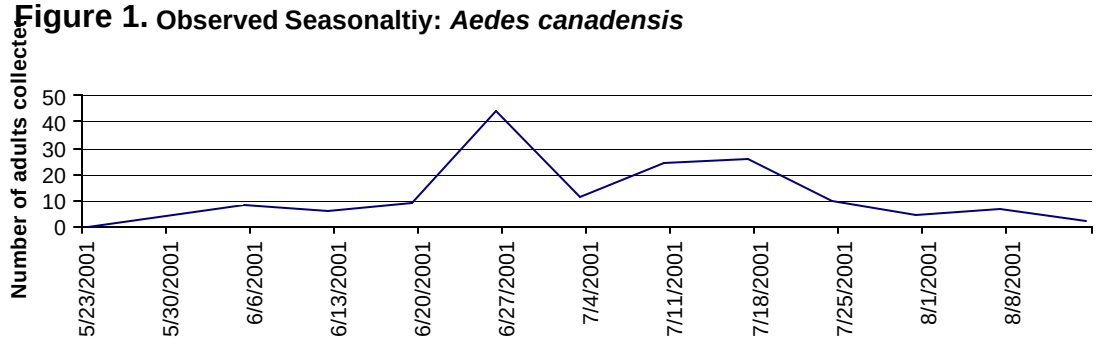
WNV Known Vector Species Collected in Portland, Maine

Aedes canadensis (Theobald)- A total of 153 adults were collected, accounting for 5.20% of the total adults collected in greater Portland (Appendix B). Most of the adult individuals (121) were trapped in a CO_2 trap at site #6 (Figure 24). Site #1 was second with eighteen individuals, sites #2, 3, 4 and #5 each had less than five individuals. None were collected using the gravid trap.

Although primarily an early season species, *Ae. canadensis,* in Portland, seemed to start about the last week of May, peak the last week of June and continue through August (Figure 1). Females prefer mammals but are also attracted to and feed on birds, amphibians and reptiles (Wood, Dang and Ellis, 148). This species is noted as having a possible second generation per season in shaded or cool locations and overwinter as eggs.

All larvae were collected from shaded leafy snowmelt water (water had a slight amber color) pools with water temperature ranging from 71? to 76? F. Both pools were dry by the end of the season. Site #6 on June 11th yielded one which was found with *Ae. cinereus* larvae. Site #1 in Baxter Woods yielded eleven larvae on June 18th. This species is listed as a major pest.

Figure 1. Observed Seasonaltiy: *Aedes canadensis*



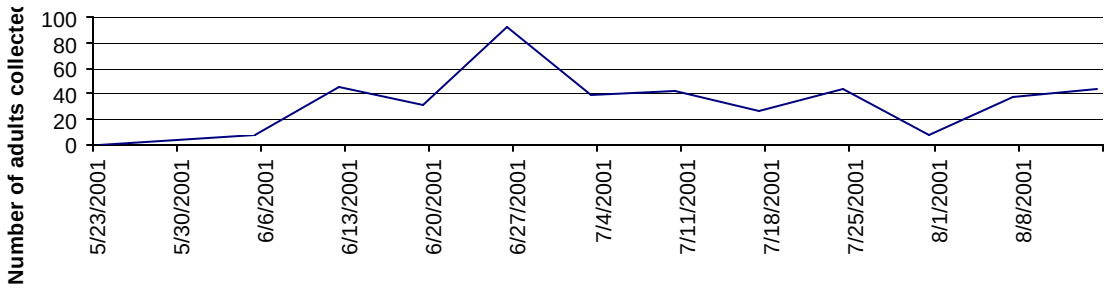
Aedes cantator (Coquillett)- A total of 417 adults were collected, accounting for 14.30% of the total adults collected in greater Portland (Appendix B). Most of the adult individuals (200) were trapped in a CO_2 trap at site #6 (Figure 24). Site #1, 3, 4 and 5 had between thirty-four and seventy individuals. Eight were collected at site #2 (Figure 20). Only one adult was collected

Aedes cantator (Coquillett)- A total of 417 adults were collected, accounting for 14.30% of the total adults collected in greater Portland (Appendix B). Most of the adult individuals (200) were trapped in a CO_2 trap at site #6 (Figure 24). Site #1, 3, 4 and 5 had between thirty-four and seventy individuals. Eight were collected at site #2 (Figure 20). Only one adult was collected using the gravid trap at site #5 on June 19th (Figure 23). *Ae. cantator,* like *Ae. sollicitans,* are coastal salt marsh and saline pool species (Wood, Dang and Ellis, 151).

Although primarily an early season species, *Ae. cantator,* in Portland, seemed to start about the last week of May, peak the last week of June and continue through August (Figure 2). They have one or more generations per year and overwinter as eggs.

Larvae of this species were not collected in Portland. *Ae. cantator* is listed as a major pest species and appears in large numbers in the evening.

Figure 2. Observed Seasonality*: Aedes cantator*



Aedes japonicus (Theobald)- Only one adult was collected from a gravid trap at site # 4 (Figure 22) on June 26th (Appendix B). **This is a new Maine record**. Peak seasonality could not be determined due to lack of sufficient specimens.

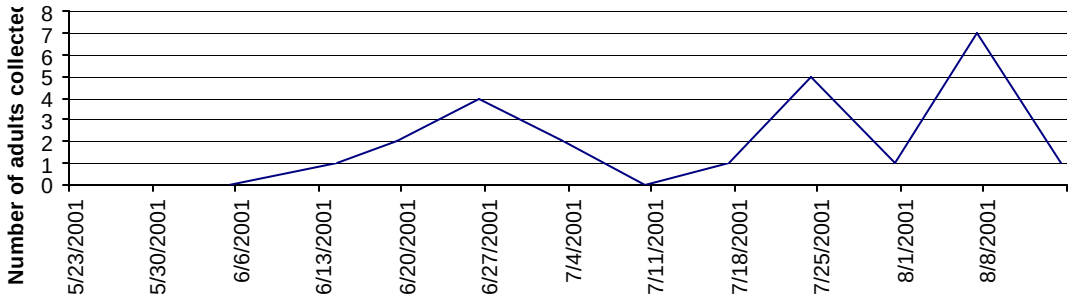
Larvae of this species were not collected in Portland during this project. However, larvae have been found in tree holes, tires, and other containers containing rich organic material. They reportedly prefer rock pools. This nonindigenous species feeds on a variety of hosts and has adapted well to northeastern climates (Fonseca, et al. 2001).

Aedes sollicitans (Walker)- A total of 23 adults were collected, accounting for 0.79% of the total adults collected in greater Portland (Appendix B). Most of the adults were collected from CO_2 traps, one was collected in a gravid trap on August 7th at site #5. Most of the adult individuals were trapped at site #4 (Figure 22) and site #5 (Figure 23). There were none collected at site #2 and the other sites had only one or two that were collected.

Ae. sollicitans adults first appeared on June 14th in Evergreen Cemetery (hand collected). The adults seemed to have several peaks throughout the season and continue through August (Figure 3). They are multivoltine and overwinter as eggs. Females are aggressive biters and carry a major pest status, however, they are not known to enter homes. They can travel long distances and will bite during the day. Adults are primarily mammalian feeders.

Although primarily salt marsh species, *Ae. sollicitans* larvae has been recorded in freshwater with *Ae. vexans* (Wood, Dang and Ellis, 246). Larvae can also develop in polluted waters. Larvae of this species were not collected in Portland during this project.

Figure 3. Observed seasonality: *Aedes sollicitans*

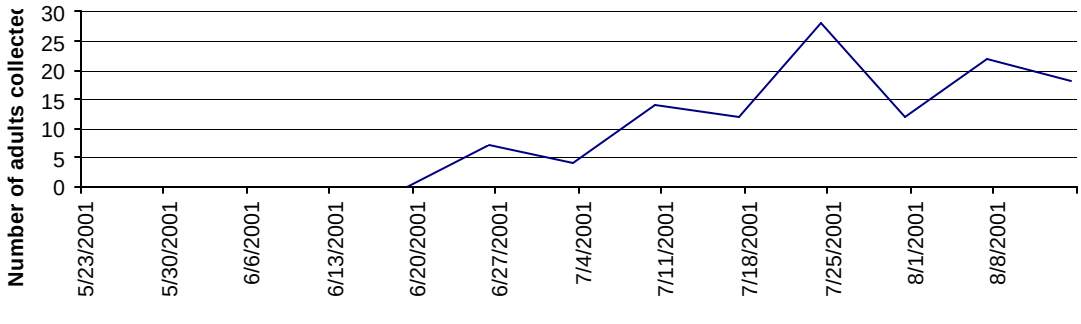


Aedes triseriatus (Say)- A total of 117 adults were collected, accounting for 4.00% of the total adults collected in greater Portland (Appendix B). Most of the adult individuals (90) were trapped in a CO_2 trap at site #6 (Figure 24). The other sites had between one and thirteen individuals. Only three were collected using the gravid trap at site #2 (Figure 20), two on August 7th and one on the 14th.

Ae. triseriatus adults first started to appear around the third week of June, peaked the fourth week of July and continued on through August (Figure 4). They overwinter as eggs, have one generation per season and larvae require a long photoperiod, of at least twelve hours, to develop (Wood, Dang and Ellis, 265). Females are active biters of a variety of hosts both day and night. *Ae. triseriatus* can also find its way into homes.

Larvae are found naturally in tree holes, but will use heavily shaded, dark containers containing rich organic material. Larvae were usually found at the very bottom of the container during this project. Larvae were collected starting on June 16th from tires located off of Lancaster Street at site #2 until August 13th. The tires were filled with maple and oak leaves and heavily shaded. The water inside was black and thick.

Figure 4. Observed Seasonality: *Aedes triseriatus*

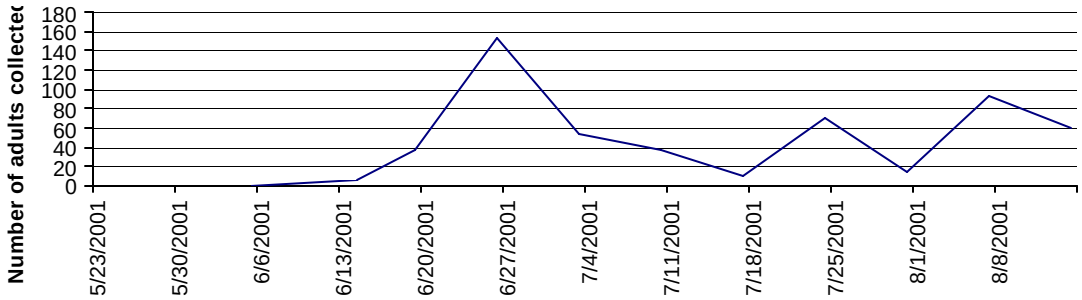


Aedes vexans (Meigen)- A total of 550 adults were collected, accounting for 18.90% of the total adults collected in greater Portland (Appendix B). Most of the adult individuals (328) were trapped in a CO_2 trap at site #6 (Figure 24). The other sites had between eighteen and fifty-eight individuals. Only two were collected using the gravid trap, one at site #2 (Figure 20) on June 26th and one at site #5 (Figure 23) on August 14th.

Ae. vexans adults first started to appear around the second week of June, peaked the fourth week of June and continued sporadically through August (Figure 5). They have two generations per season and overwinter as eggs. With a moderate pest status, *Ae. vexans* females will bite during the day but is most active during the evening and at night.

A single *Ae. vexans* larvae was collected at Site #6 on June 11th and was found with *Ae. cinereus* and *Ae. canadensis* larvae in a shaded leafy melt water (water had a slight amber color) pool. In late July and August forty-one *Ae. vexans* individuals were collected from a shallow puddle that filled each time it rained in the parking lot at Portland's Bayside Park along with the larvae of *Ae. trivittatus* and *Cx. pipiens.* The puddle had a grassy edge and a gravel bottom.

Figure 5. Observed seasonality: *Aedes vexans*

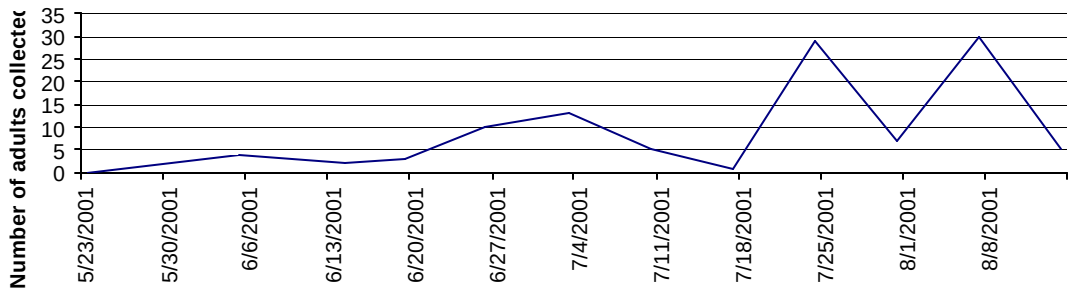


Anopheles punctipennis (Say)- A total of 109 adults were collected, accounting for 3.70% of the total adults collected in greater Portland (Appendix B). Most of the adult individuals (67) were trapped in a CO_2 trap at site #6 (Figure 24). The other sites had between one and seventeen individuals. Only one was collected at site #2 (Figure 20) using the gravid trap on August 7th.

An. punctipennis adults first started to appear around the first week of June, and had several peaks from June through August (Figure 6). They are multivoltine and overwinter as adults.

Larvae were collected during the Portland project, and were found primarily in ponds at sites #1 and #6 with *Cx. territans* as early as June, 9th. In Lewiston, Maine, larvae were collected in muddy, semi-shaded puddles and in the rusty water of an old metal cooking pot.

Figure 6. **Observed seasonality:** ***Anopheles punctipennis***

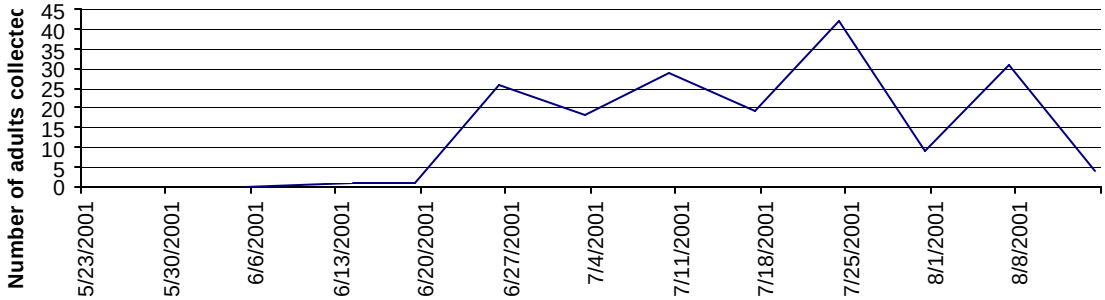


Coquillettidia perturbans (Walker)- A total of 179 adults were collected, accounting for 6.10% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from CO_2 traps. Most of the adult individuals (155) were trapped at site #6 (Figure 24). Sites #1, 3, 4 and #5 contained anywhere from two to thirteen adults. None were trapped at site #2 (Figure 20).

Cq. perturbans adults first started to appear around the second week of June, and have several peaks from June through August (Figure 7). As a major pest status, they will bite day and night and can also find their way into homes. Females feed primarily on birds and mammals including humans (Wood, Dang and Ellis, 319). They are reportedly univoltine but seemed to have multiple generations during this season. They overwinter as larvae.

Larvae of this species were not collected in Portland during this project but should be found attached to the bottom of aquatic plants (Wood, Dang and Ellis, 318).

Figure 7. **Observed seasonality:** ***Coquillettidia perturbans***



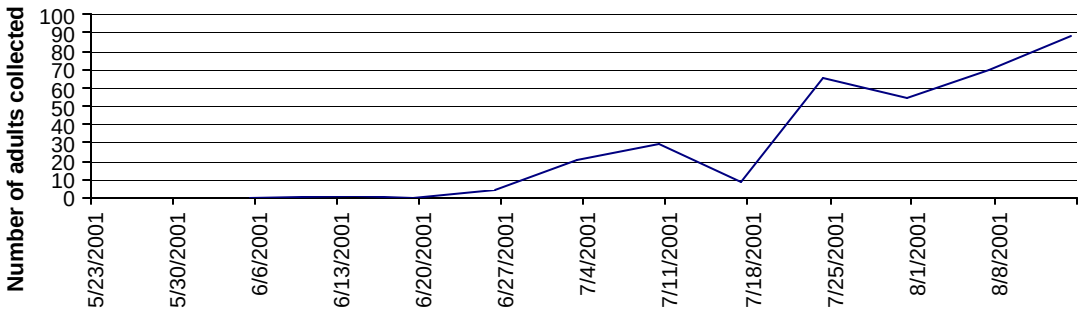
Culex pipiens (Linn.)- A total of 340 adults were collected, accounting for 11.70% of the total adults collected in greater Portland (Appendix B). Adults were collected from both CO_2 and gravid traps. *Cx. pipiens* were found to be abundant throughout greater Portland. Sites #2, 3 and #5 ranged from 81 to 98 adults trapped, with the other sites ranging from sixteen to thirty-two.

Cx. pipiens adults first started to appear around the second week of June, and gradually increased through August (Figure 8). Females feed primarily on birds and will bite small mammals, snakes and turtles. It is not known if they bite humans (Wood, Dang and Ellis, 276). They are multivoltine and overwinter as adults.

Egg raft sampling for *Cx. pipiens* resulted in 166 rafts collected, starting on July 3rd. The majority of rafts were collected after the third week of July and appeared to overlap with *Cx. restuans* rafting. An apparent increase in the numbers of individual rafts was noticeable after periods of very hot, dry weather.

Larvae were found as early as June 25th at Bayside Park in a large flooded grassy depression (used as an ice rink in winter) along with *Cx. restuans* and *Cx. salinarius*. On the same day they were also collected with *Cx. restuans* in a plastic overturned trash lid filled with water (water was an amber color from oak and maple leaves on bottom). In the early season *Cx. pipiens* seemed to prefer containers filled with water, a dirt bottom and emergent vegetation. Throughout the season, *Cx. pipiens* could be found in almost any artificial or natural container of any color or type in association with *Cx. restuans*. Some places, such as the Maggie Circle development (site #6) they were found crowded with *Cx. territans*, *An. punctipennis* and *Ur. sapphirina* in a shaded permanent pool, with emergent and decaying vegetation, created by construction. At site #1, they were collected in a metal washtub, filled with organic debris and rainwater, along with *Cx. restuans* and *Cx. salinarius.*

Figure 8. Observed seasonality: *Culex pipiens*



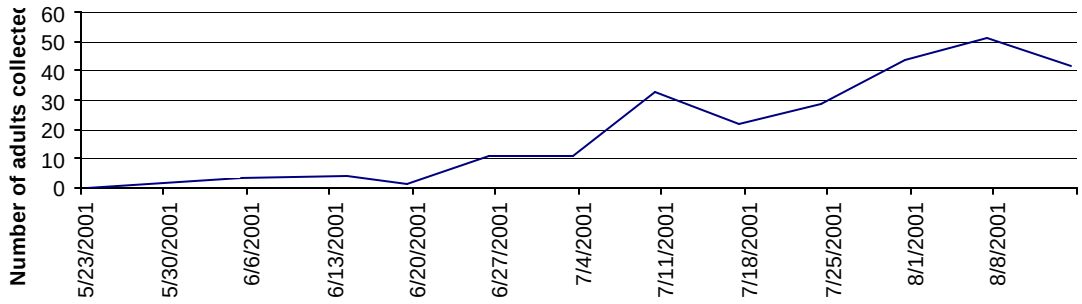
Culex restuans (Theobald)- A total of 251 adults were collected, accounting for 8.60% of the total adults collected in greater Portland (Appendix B). Adults were collected from both CO_2 and gravid traps. *Cx. restuans* adults were found to be abundant throughout greater Portland ranging from eight (site #4) to seventy eight (site #2).

Cx. restuans adults first started to appear slightly before *Cx. pipiens*, around the first week of June, and had several peaks from June through August (Figure 9). Females feed at night, primarily on birds and mammals and are known to bite humans (Wood, Dang and Ellis, 279). They are multivoltine and overwinter as adults.

Egg raft sampling for *Cx. restuans* resulted in 554 rafts collected starting on July 3rd, and continued throughout the season.

Larvae were found as early as June 4th, in most of the same places as *Cx. pipiens.* Through the season, *Cx. restuans* larvae were found in almost any artificial or natural container of any color or type. They were found in shaded cement cemetery urns, in mud puddles on the direct edge of the pond at Baxter Woods (site #1). The pond itself was void of any larvae. They were even found in a five gallon bucket filled with water that was once used for roofing tar (site #2). The tar bucket still had a partial petroleum scum left on the surface of the water. In some places, such as Maggie Circle development (site #6), they were found crowded with *Cx. territans*, *An. punctipennis* and *Ur. sapphirina* in a shaded permanent pool, with emergent and decaying vegetation, created by construction. At times they shared containers with *Ae. triseriatus*.

Figure 9. **Observed seasonality: *Culex restuans***

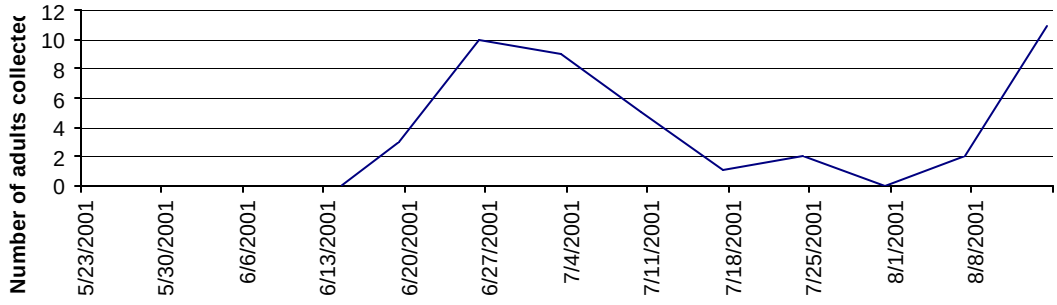


Culex salinarius (Coquillett)- A total of 43 adults were collected, accounting for 1.50% of the total adults collected in greater Portland (Appendix B). Although adults were collected from both CO_2 and gravid traps, only two individuals were collected from a gravid trap at site #2 (Figure 20). *Cx. salinarius* adults were collected at all sites except site #4 (Figure 22). At sites #3 and #6, CO_2 traps collected seventeen and thirteen individuals, respectively. Site #6 (Figure 24) collected the first adult in a CO_2 trap on June 19th. The other sites ranged from two to seven adults.

Culex salinarius adults first started to appear around the third week of June, and had several peaks from June through August (Figure 10). Females are indiscriminate feeders and are known to bite humans. They are multivoltine and overwinter as adults.

Larvae were first collected on June 25th at Bayside Park (site #1) along with *Cx. restuans* and *Cx. pipiens* in a large flooded grassy depression (used as an ice rink in winter). On the same day, they were collected from a large metal washtub (site #1) along with *Cx. restuans* and *Cx. pipiens*. *Cx. salinarius* larvae were also collected at Maggie Circle development (site #6), they were found crowded with *Cx. territans*, *Cx. restuans* and *Cx. pipiens* in a shaded permanent pool, with emergent and decaying vegetation, created by construction. Larvae were collected until July 31st.

Figure 10. **Observed seasonality: *Culex salinarius***



Culiseta melanura (Coquillett)- Two adults were collected (Appendix B), one on July 10th at site #3 (Figure 21) and the other on August 14th at site #4 (Figure 22). Both were collected from CO_2

traps. Peak seasonality could not be determined due to lack of sufficient specimens. *Cs. melanura* has several generations per season. Females prefer birds as hosts and will rarely bite humans.

Larvae of this species were not collected in Portland during this project but should be found in freshwater swamps, artificial and natural containers and ground pools under trees (Wood, Dang and Ellis, 305).

Other Species Collected in Portland, Maine

Aedes abserratus (Felt & Young)- Only four adults (Appendix B) were collected from a CO_2 trap at site #6 (Figure 24) on June 19th and June 26th. *Ae. abserratus* overwinters as eggs and is a univoltine, spring species. Peak seasonality could not be determined due to lack of sufficient specimens.

Larvae of this species were not collected in Portland during this project.

Aedes atropalpus (Coquillett)- Only one adult (Appendix B) was collected from a CO_2 trap at site #3 (Figure 21) on July 3rd. *Ae. atropalpus* overwinters as eggs and is a univoltine, spring species. Peak seasonality could not be determined due to lack of sufficient specimens.

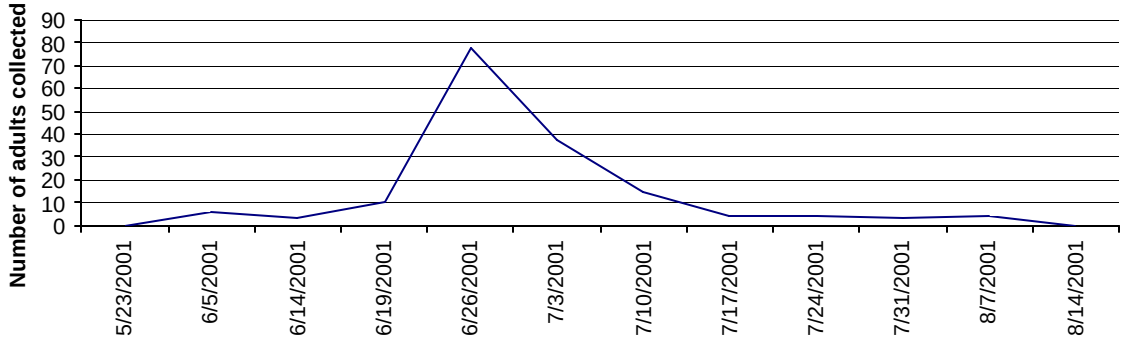
Larvae of this species were not collected in Portland during this project.

Aedes cinerus (Meigen)- A total of 162 adults were collected, accounting for 5.60% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from CO_2 traps except two from gravid traps. Most of the adult individuals (109) were trapped at site #6 (Figure 24). None were trapped at site #5 (Figure 23).

Ae. cinerus adults first started to appear around the first week of June, and peaked around the last week of June and continued to the first week of August (Figure 11). They may have more than one generation per season and overwinter as eggs. Females are active at night and will feed on humans (Wood, Dang and Ellis, 158).

Larvae were collected with *Ae. canadensis* and *Ae. vexans* from wooded temporary snowmelt pools at site #6 on June 11th.

Figure 11. Observed seasonality: *Aedes cinereus*



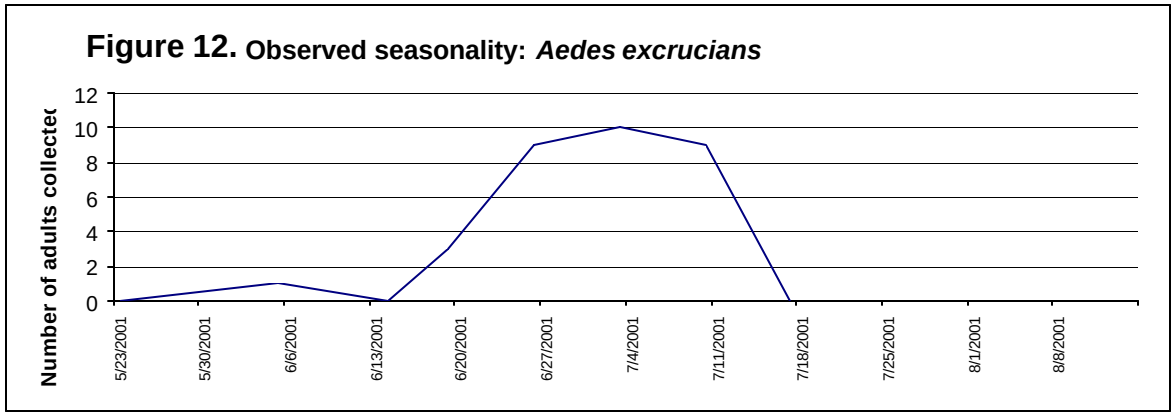
Aedes communis (DeGeer)- Only four adults (Appendix B) were collected from a CO_2 trap at site #6 (Figure 24) from June 26th to July 10th. *Ae. communis* overwinters as eggs and is a spring species. Peak seasonality could not be determined due to lack of sufficient specimens. They are univoltine and overwinter as eggs.

Larvae of this species were not collected in Portland during this project.

Aedes excrucians (Walker)- A total of 32 adults were collected, accounting for 1.10% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from CO_2 traps. Most of the adult individuals (18) were trapped at site #6 (Figure 24). None were trapped at site #4 (Figure 22).

Ae. excrucians adults, a spring species, first started to appear the last week of May, and peak around the first week of June and continue into the second week of July (Figure 12). They are univoltine and overwinter as eggs. Females will feed on humans (Wood, Dang and Ellis, 176).

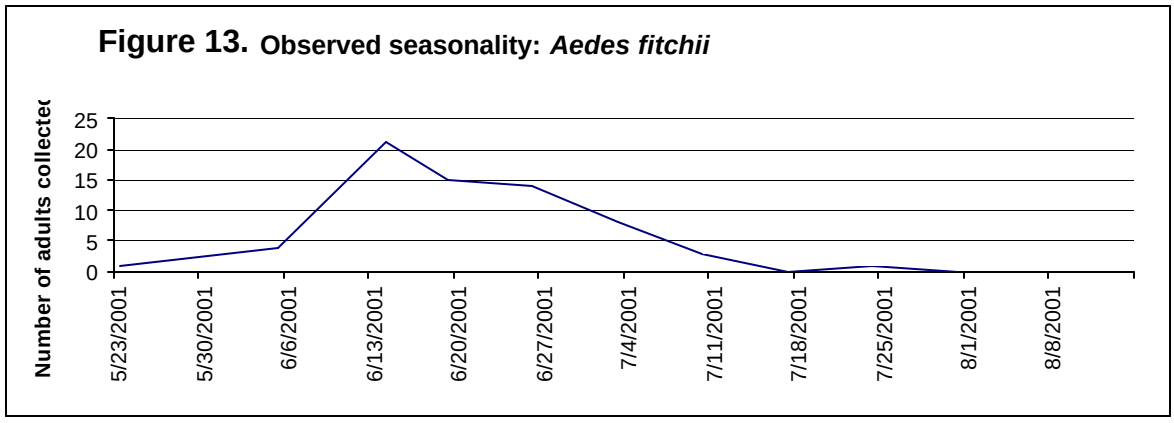
Larvae of this species were not collected in Portland during this project but have been found in snow pools with other spring species.



Aedes fitchii (Felt & Young)- A total of 67 adults were collected, accounting for 2.30% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from CO_2 traps. Most of the adult individuals were trapped at sites #6 (Figure 24) and #3 (Figure 21).

Ae. fitchii adults, a spring species, first appeared on May 23rd at site #3. They seemed to peak around the third week of June and continued into the last week of July (Figure 13). They are univoltine and overwinter as eggs. (Wood, Dang and Ellis, 180).

Larvae of this species were not collected in Portland during this project but have been found in snow pools and usually associated with *Ae. excrucians.*



Aedes intrudens (Dyar)- Only one adult (Appendix B) was collected from a CO_2 trap at site #6 (Figure 24) on June 5th. Peak seasonality could not be determined due to lack of sufficient specimens. *Ae. intrudens* overwinters as eggs, and are early species with one generation per season.

Larvae develop in temporary woodland snowmelt pools. Females will bite humans. However, not a known pest species because season is short lived (Wood, Dang and Ellis, 206). Larvae of this species were not collected in Portland during this project.

Aedes provocans (Walker)- Five adults (Appendix B) were collected. One adult was hand collected on June 11th at site #2 (Figure 20) , four from a CO_2 trap at site #6 (Figure 24). Two of these, from site #6 were collected on June 19th and the other two on June 26th. Peak seasonality could not be determined due to lack of sufficient specimens. They overwinter as eggs, are early season species with one generation and are a minor pest.

Ae. provocans larvae develop in temporary woodland snowmelt pools, semi permanent marshes or in roadside ditches (Wood, Dang and Ellis, 225). Larvae of this species were not collected in Portland during this project.

Aedes punctor (Kirby)- A total of 13 adults were collected, accounting for 0.45% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from CO_2 traps. Most of the adult individuals were trapped at site #6 (Figure 24). None were collected at sites #2, #4 and #5.

Ae. punctor adults, a spring species, first appeared on June 5th at site #6. The adults seemed to peak before the third week of June and continued into the second week of July (Figure 14). They are univoltine and overwinter as eggs.

Larvae can be found in snow pools, sphagnum bogs and open grassy areas. They can be associated with *Ae. abserratus* and *Ae. cinereus* larvae (Wood, Dang and Ellis, 232). Larvae of *Ae. punctor* were not collected in Portland during this project.

Figure 14. Observed seasonality: *Aedes punctor*

Aedes sticticus (Meigen)-Five adults (Appendix B) were collected throughout Portland in the month of June. They were all collected in CO_2 traps at sites #6, 4, 3 and #1. Peak seasonality could not be determined due to lack of sufficient specimens.

Ae. sticticus can have one or more generations per season dependant on flooding of river plains or bottomlands, but they usually occur in late spring after snowmelt. Eggs can remain viable for five years and are not temperature dependant for hatching. Pest status is local and can be major, species can also be nonexistent for a season (Wood, Dang and Ellis, 251).

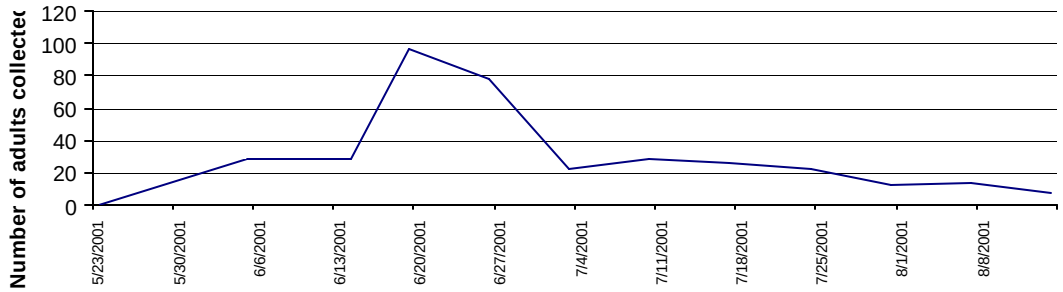
Larvae of this species were not collected in Portland during this project.

Aedes stimulans (Walker)- A total of 365 adults were collected, accounting for 12.50% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from CO_2 traps. Most of the adult individuals were trapped at site #6 (Figure 24). None were collected at sites #3 (Figure 21) and between one and three were collected at the other sites.

Ae. stimulans adults, a spring species, first appeared on June 4th at site #2. The adults seemed to peak before the third week of June and continued through August (Figure 15). They are univoltine and overwinter as eggs and are a long lived, major pest species.

Larvae of this species were not collected in Portland during this project, however, they have been found in wooded floodplains with spring runoff (Wood, Dang and Ellis, 256).

Figure 15. Observed seasonality: *Aedes stimulans*

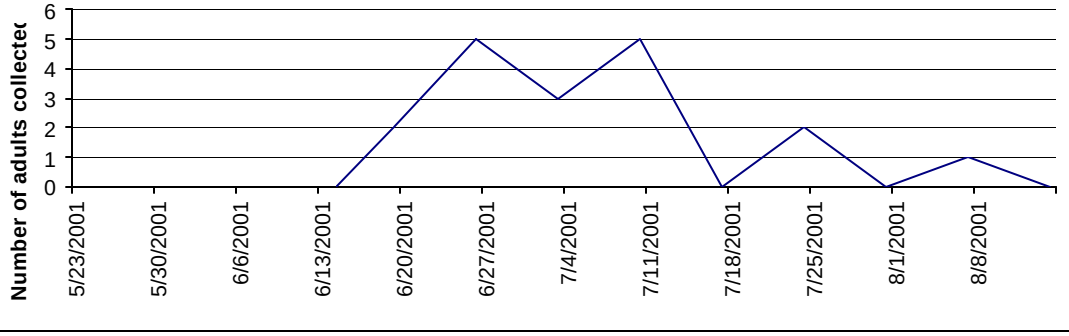


Aedes trivittatus (Coquillett)- A total of 18 adults were collected, accounting for 0.62% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from CO_2 traps. Most of the adult individuals were trapped at sites #1 (Figure 24) and #6 (Figure 24). None were collected at sites #2, #3 and #4.

Ae. trivittatus adults, a late spring species, first appeared on June 19th at site #1. The adults seemed to have several peaks and continued into August (Figure 16). They are early spring species, have one generation per season and overwinter as eggs. They are active day and night biters and will feed on humans (Wood, Dang and Ellis, 268).

Larvae of *Ae. trivittatus* were collected in June in a flooded grassy depression with *Cx. pipiens*. They were also collected in late July and August from a shallow puddle, that filled each time it rained, in the parking lot along with the larvae of *Ae. vexans* and *Cx. pipiens*. Both collections took place at Portland's Bayside Park.

Figure 16. Observed seasonality: *Aedes trivittatus*

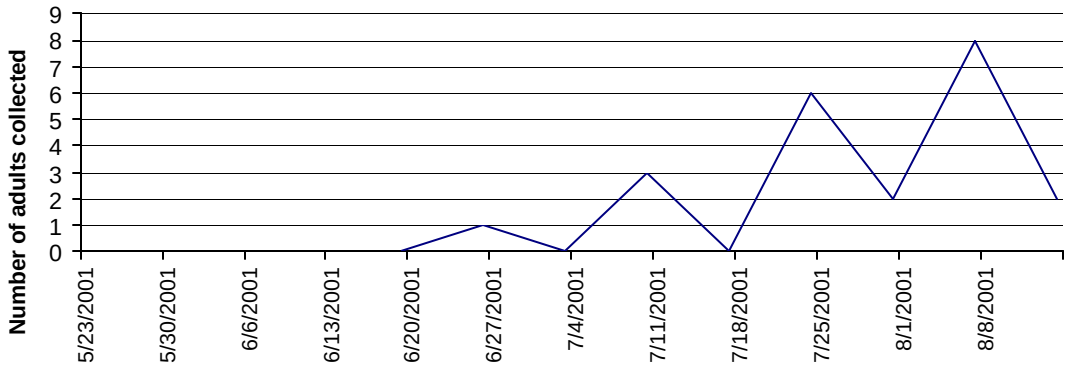


Anopheles quadrimaculatus (Say)- A total of 22 adults were collected, accounting for 0.76% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from CO_2 traps. Most of the adult individuals were trapped at site #6 (Figure 24). None were collected at site #2 (Figure 20). The other sites each had one adult trapped.

An. quadrimaculatus adults first appeared on June 25th at site #6. The adults seemed to have several peaks and continue through August (Figure 17). They overwinter as adults. Females are primarily night biters, they will feed on humans.

Larvae were collected mainly from ponds starting on June 19th. Larvae of *An. quadrimaculatus* can also be found in fresh and salt water marshes (Wood, Dang and Ellis, 86).

Figure 17. Observed seasonality: *Anopheles quadrimaculatus*



Culex territans (Walker)- Five adults (Appendix B) were collected, starting on July 3rd then throughout the season, in Portland at sites #2, 4 and #5. Three were collected in a gravid trap, two in a CO_2 trap. This species is multivoltine, overwinters as adults, and prefers to feed on amphibians and reptiles. Peak seasonality could not be determined due to lack of sufficient specimens.

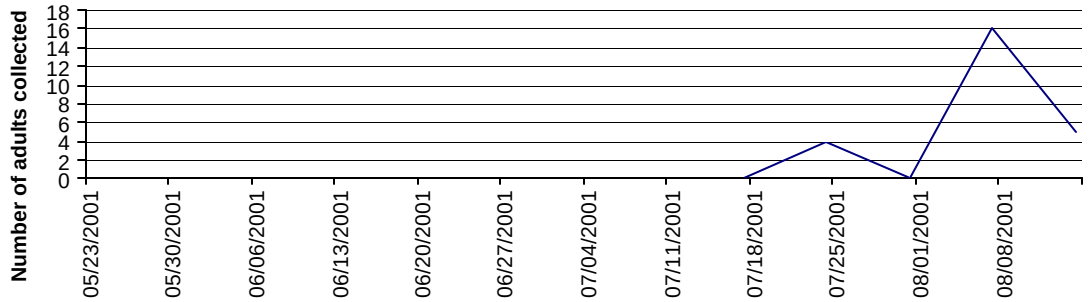
Cx. territans larvae were found with *Anopheles spp*., starting on May 18th, primarily on the edges of ponds and fresh water marshes. Later in the season, in August, they were collected, with *Cx. pipiens* and *Cx. restuans,* in tires (site #2) and in standing water in poured foundations at Maggie Circle (site #6).

Culiseta morsitans (Coquillett)- Two adults (Appendix B) were collected at site #1 from the CO_2 trap, one on June 12th and the other on July 31st. Peak seasonality could not be determined due to lack of sufficient specimens. *Cs. morsitans* overwinters as eggs, are multivoltine and long lived. Females feed primarily on birds and will also feed on small mammals and snakes (Wood, Dang and Ellis, 313). Larvae of this species were not collected in Portland during this project

Uranotaenia sapphirina (Osten Sacken)- A total of 25 adults were collected, accounting for 0.86% of the total adults collected in greater Portland (Appendix B). All of the adults were collected from the CO_2 trap at site #6 (Figure 24). *Ur. sapphirina* adults first appeared on July 24th and continued through August (Figure 18).

Larvae were collected on August 13th at Maggie Circle development (site #6). They were found with *Cx. territans*, *Cx. restuans*, *Cx. pipiens* and *An. punctipennis* in a shaded permanent small pool, with emergent and decaying vegetation, created by construction.

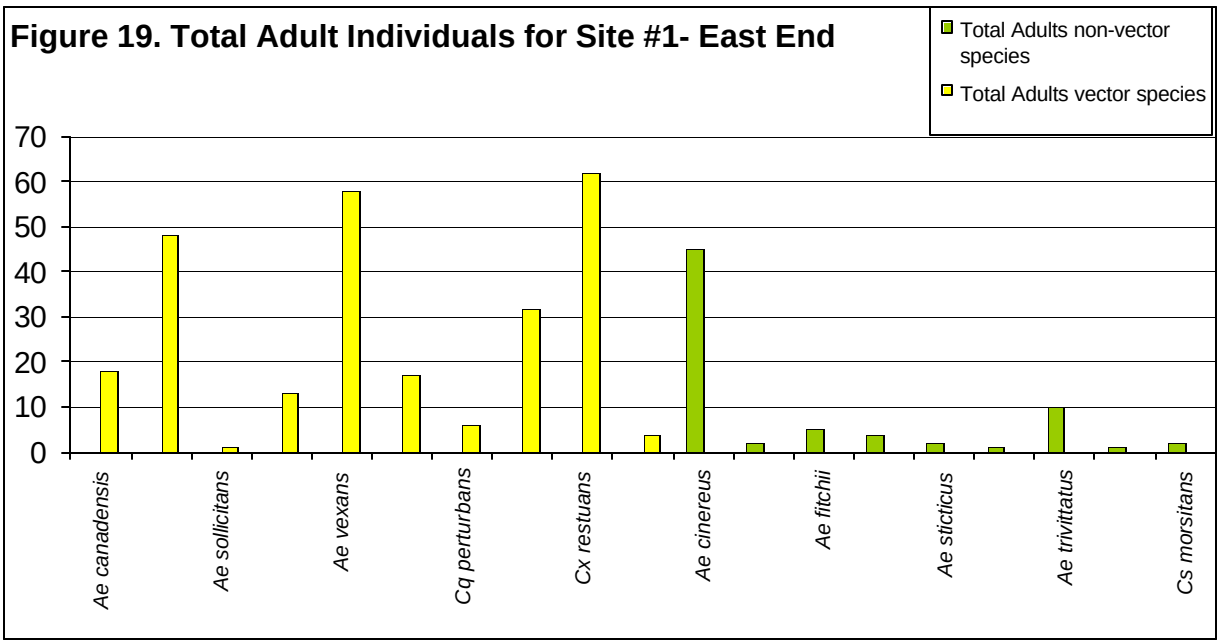
Figure 18. Observed seasonality: *Uranotaenia sapphirina*



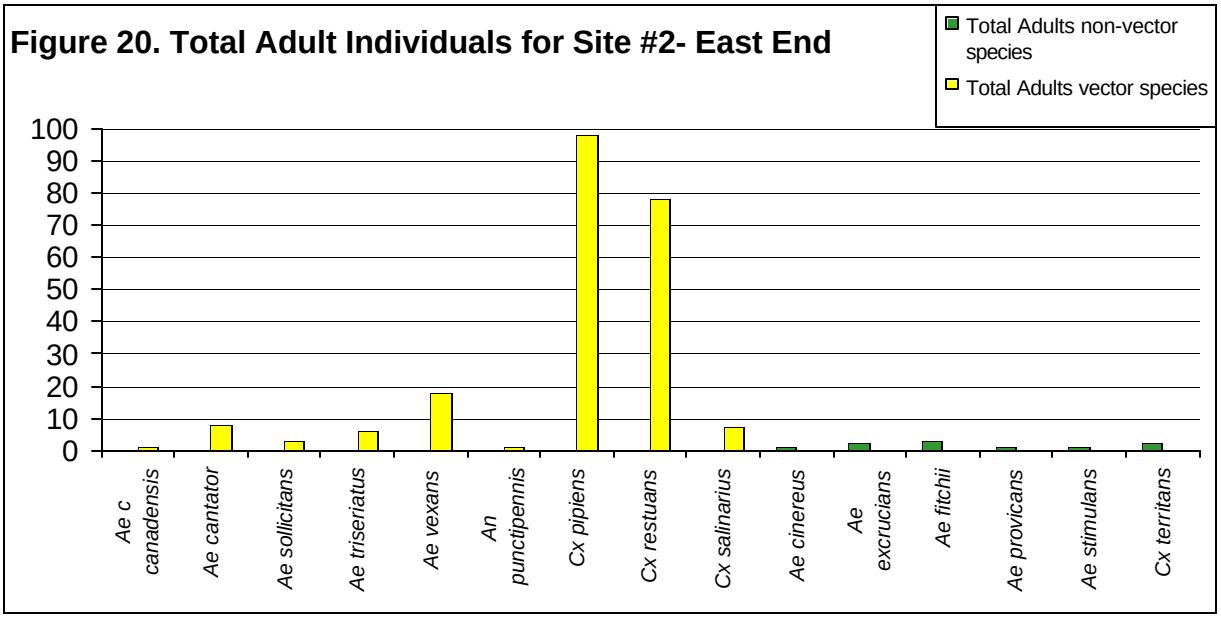
Adult Mosquito Species Collected By Site for Portland, Maine

Site #1- East Deering and Deering trapping resulted in 18 species and 329 individual adults (Figure 19). Larvae of nine species were found within this area of Portland: *Ae. canadensis, Ae. trivittatus, Ae. vexans, Cx. pipiens, Cx. restuans, Cx, salinarius, Cx. territans, An. punctipennis,* and *An. quadrimaculatus.* The most commonly collected adult species were *Ae. cantator, Ae. cinereus, Ae. vexans, Cx. pipiens* and *Cx. restuans.* Site #1 contained 11.30% of the total adults collected in Portland.

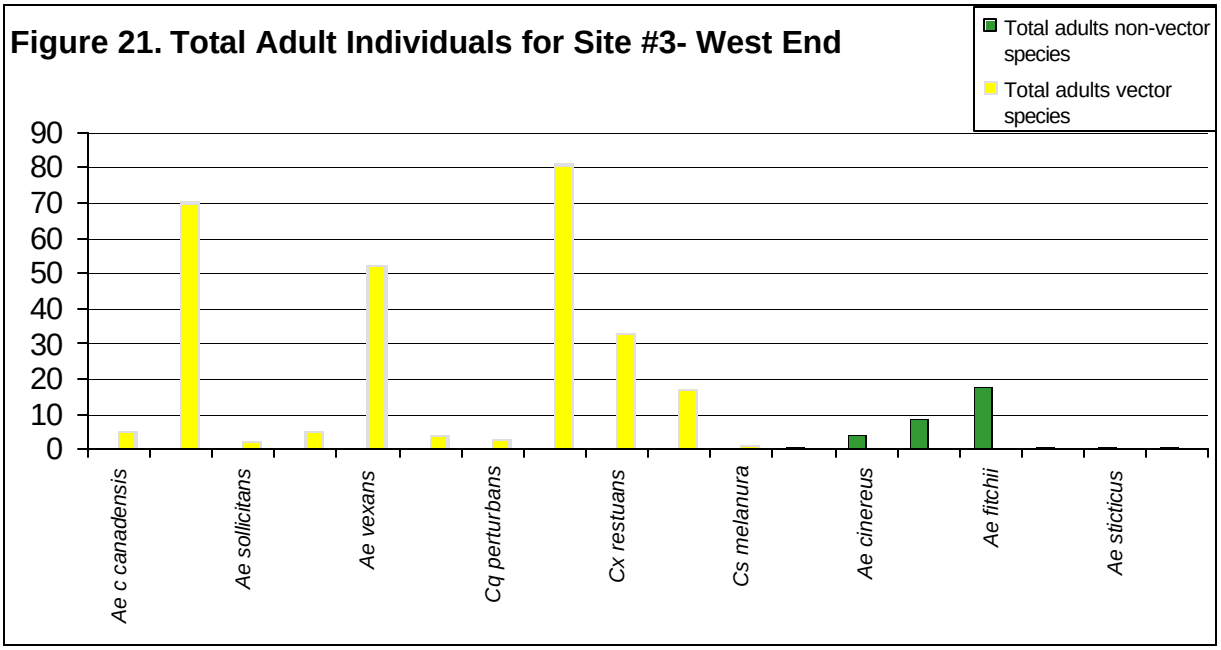
Figure 19. Total Adult Individuals for Site #1- East End



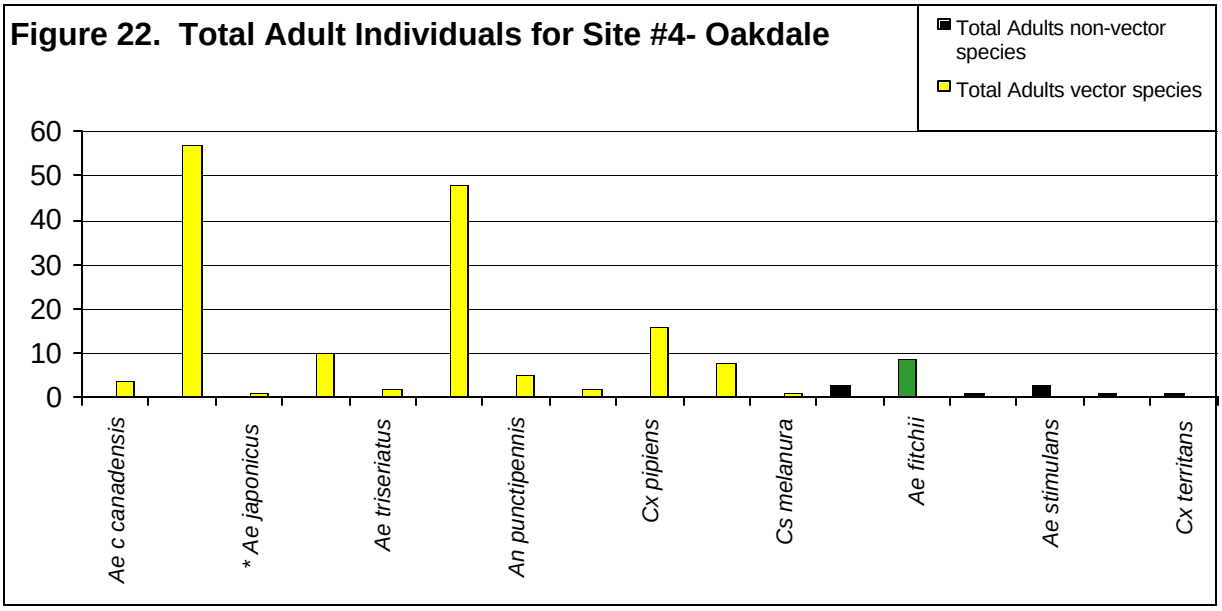
Site #2- East End trapping resulted in 15 species and 230 individual adults (Figure 20). Larvae of three species were found within this area of Portland: *Ae. triseriatus, Cx. pipiens, Cx. restuans,* and *Cx. territans.* The most commonly collected adult species were *Cx. pipiens* and *Cx. restuans.* Site #2 contained 7.90% of the total adults collected in Portland.



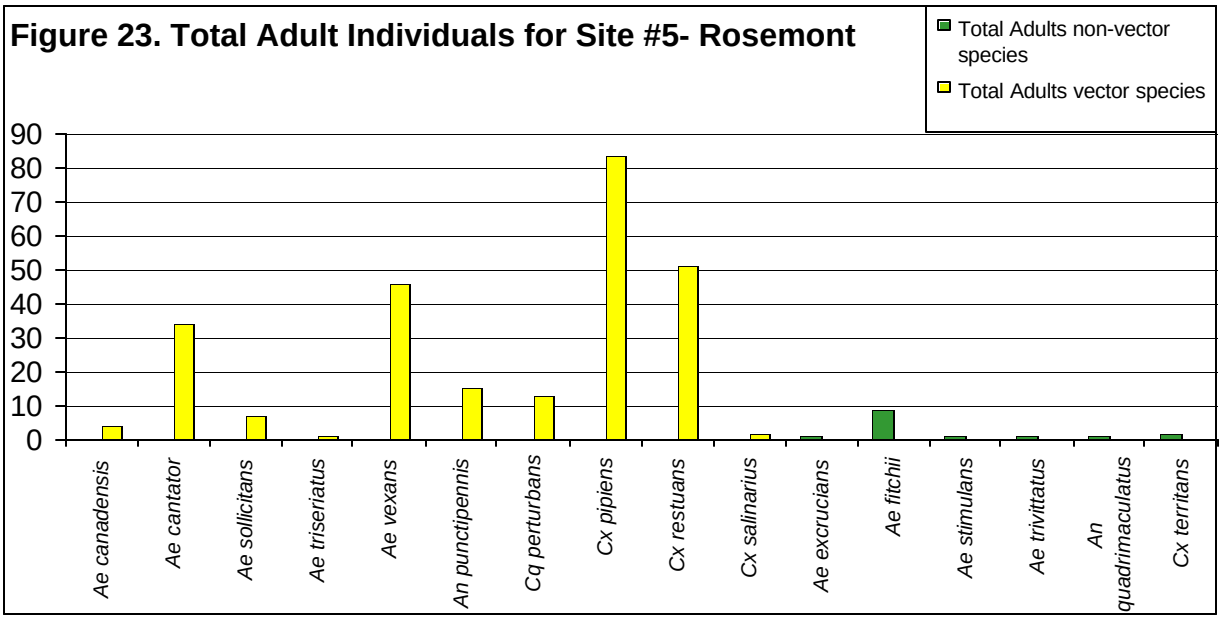
Site #3- West End trapping resulted in 18 species and 308 individual adults (Figure 21). Larvae of four species were found within this area of Portland: *Cx. pipiens, Cx. restuans, Cx, salinarius,* and *An. quadrimaculatus.* The most commonly collected adult species were *Ae. cantator, Ae. vexans, Cx. pipiens* and *Cx. restuans.* Site #3 contained 10.60% of the total adults collected in Portland.



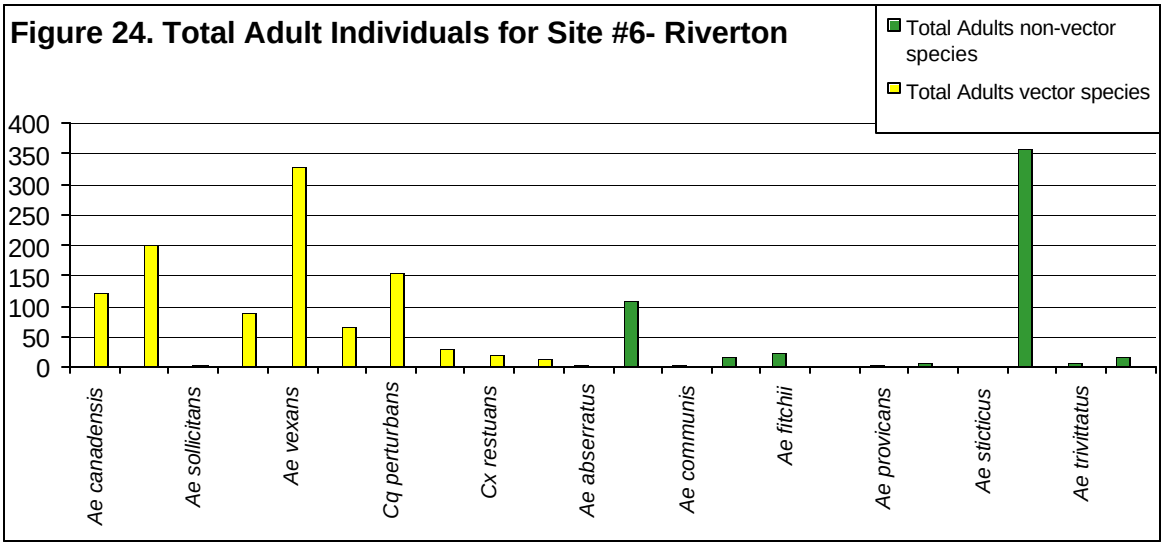
Site #4- Oakdale trapping resulted in 17 species and 172 individual adults (Figure 22). * **An *Aedes japonicus* adult, a new Maine record, was collected in a gravid trap at this site.** Larvae of *Cx. pipiens, Cx. restuans* were found within this area of Portland. The most commonly collected adult species were *Ae. cantator, Ae. vexans* and *Cx. pipiens*. Site #4 contained 5.90% of the total adults collected in Portland.



Site #5- Rosemont trapping resulted in 16 species and 271 individual adults (Figure 23). Larvae of three species were found within this area of Portland: *Cx. pipiens, Cx. restuans* and *Cx. territans*. The most commonly collected adult species were *Ae. cantator, Ae. vexans, Cx. pipiens* and *Cx. restuans.* Site #5 contained 9.30% of the total adults collected in Portland.



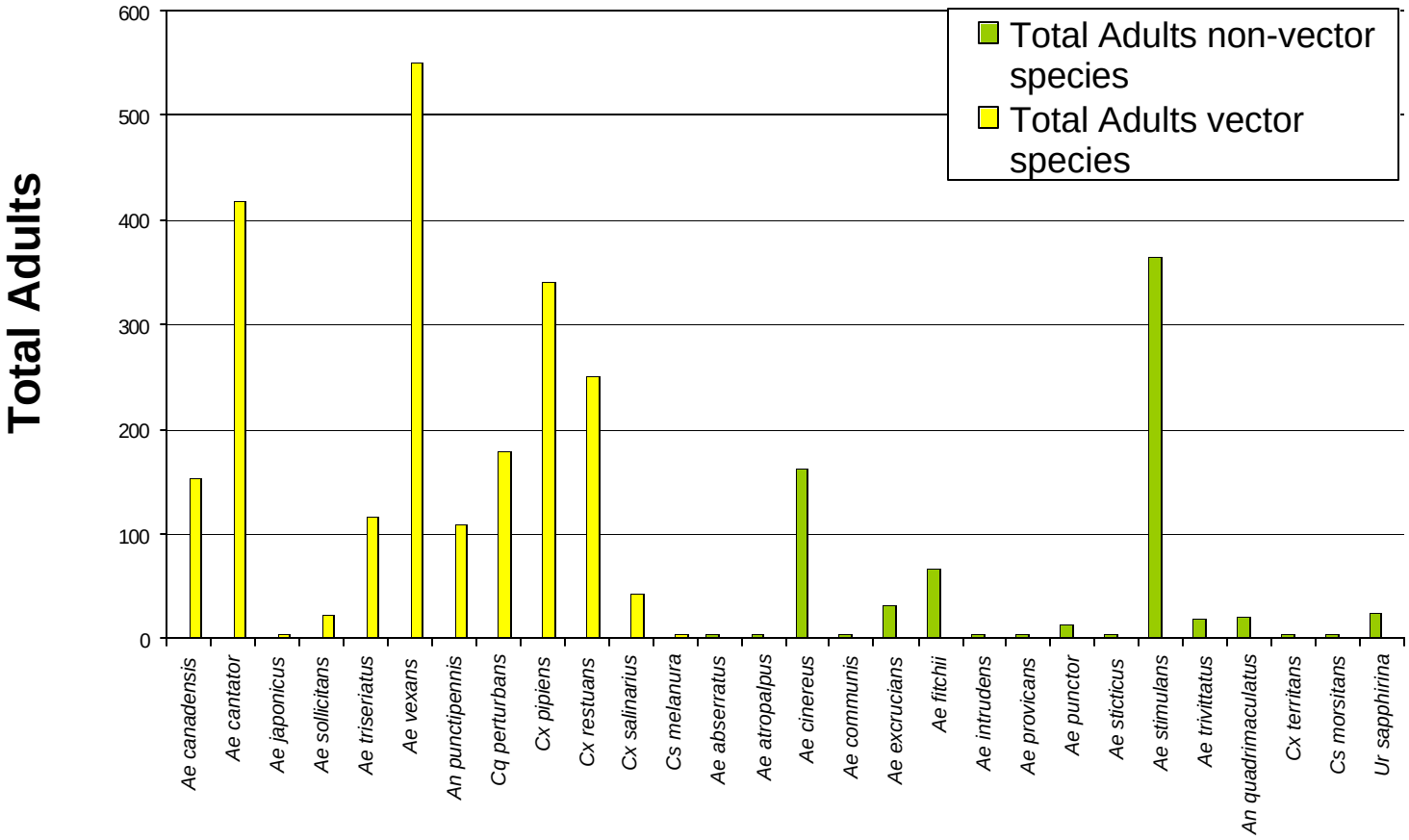
Site #6- Riverton trapping resulted in 23 species and 1,606 individual adults (Figure 24). Larvae of ten species were found within this area of Portland: *Ae. canadensis, Ae. cinereus, Ae. vexans, Cx. pipiens, Cx. restuans, Cx, salinarius, Cx. territans, An. punctipennis, Ur. sapphirina* and *An. quadrimaculatus.* The most commonly collected adult species were *Ae. canadensis, Ae. cantator, Ae. cinereus, Ae. stimulans, Ae. vexans, Cq. perturbans.* Site #6 contained 55.10% of the total adults collected in Portland.



Total Mosquitoes Collected in Greater Portland, Maine

Twenty-eight species of mosquitoes were collected and identified during this sampling project in the greater Portland. This is approximately 70% of the 40 species now known to occur in Maine (Figure 25). Twelve of the species are known WNV vector species (Appendix B). During the project season, 2,916 individual adults were collected and identified. The most commonly occurring species were *Ae. vexans, Cx. pipiens, Cx. restuans, Ae. cantator and Ae. stimulans*. Only one *Ae. japonicus* was collected. Over 4,700 larvae were collected and twelve of the twenty-eight species were identified in the larval stage (Appendix C). Seven of the larval species that were collected are known WNV vector species (Appendix B). Egg raft sampling resulted in collection of 1,004 individual rafts, large numbers of which came from site #2. Viable rafts resulted in 554 *Cx. restuans* and 166 *Cx. pipiens* rafts.

Figure 25. Total Adults Trapped in Portland, Maine



Conclusions

During the project season it became apparent there were several areas in Portland where potential vector species of mosquitoes and their larvae were more prevalent. Sites #2 and #6 are of high concern because of large human populations and the availability of manmade mosquito breeding sites available for WNV vector species. Site #6 was an active adult site and had the highest count of adult mosquitoes and a broader variety of larval species, including vector species. At this site the natural flow of water had been altered by construction. Instead of draining in the late spring and drying in the summer, it now had permanent pools of water with a high organic content bordering the property. This site will be full of multifamily units when complete. Site #2 represents the Munjoy Hill area, the Portland wastewater treatment facility and the

Eastern Promenade walkway. This site is important based on its human population and proximity to recreational areas.

Although particularly dry this summer, mosquito larvae could still be found in abundance. Many of the *Culex* species shared containers with the *Aedes* species. It seemed there were several criteria needed for survival; water, shade, lack of predators, amount of daylight and/or temperature. No larvae were found in cement containers (birdbaths) that were not shaded from the sun. Even blades of grass in an unsheltered bucket *(Cx. pipiens)*, a rock in a puddle (*Ae. vexans*), a bucket in an alley (*Cx. restuans*) or the upper side of an old tire (*Ae. triseriatus*) offered enough shade for larvae to gather in the shaded areas. Ponds do not seem to contain many species, there are too many predators. A small indent from a vehicle tire, filled with water and three inches from a pond, was enough to protect the developing larvae of *Cx. restuans* from predators. Female mosquitoes laid eggs in that puddle but did not in the pond, which was only inches away. *Anopheles spp.* are common in ponds because they can stay on the surface within floating weeds, unnoticed by potential predators.

For some species, a difference in water quality, type of organic material in the water and color of the container may have determined where larvae developed. Some species preferred fairly clear water with overhanging vegetation, some preferred dark, amber colored water with a high content of leaf debris and others preferred the lighter colored hay infusion. It would be interesting to find out what different species prefer as an egg laying medium: clear shaded water with emergent grass, maple/oak leaf infusion, algae blooms, or the hay infusion. For adult female mosquito species, the CO_2 trap used a light as well as dry ice as an attractant. There is also the need to identify which attractant is most effective for certain species; the light, the CO_2, both or neither. Further research is needed to define the nature of Maine's mosquito populations. For mosquito control we need to further refine answers to questions such as;

1) What is the importance of each of our Maine species?
2) Where do they breed?
3) When are they active in each stage?
4) How do we best respond to the need for population management?

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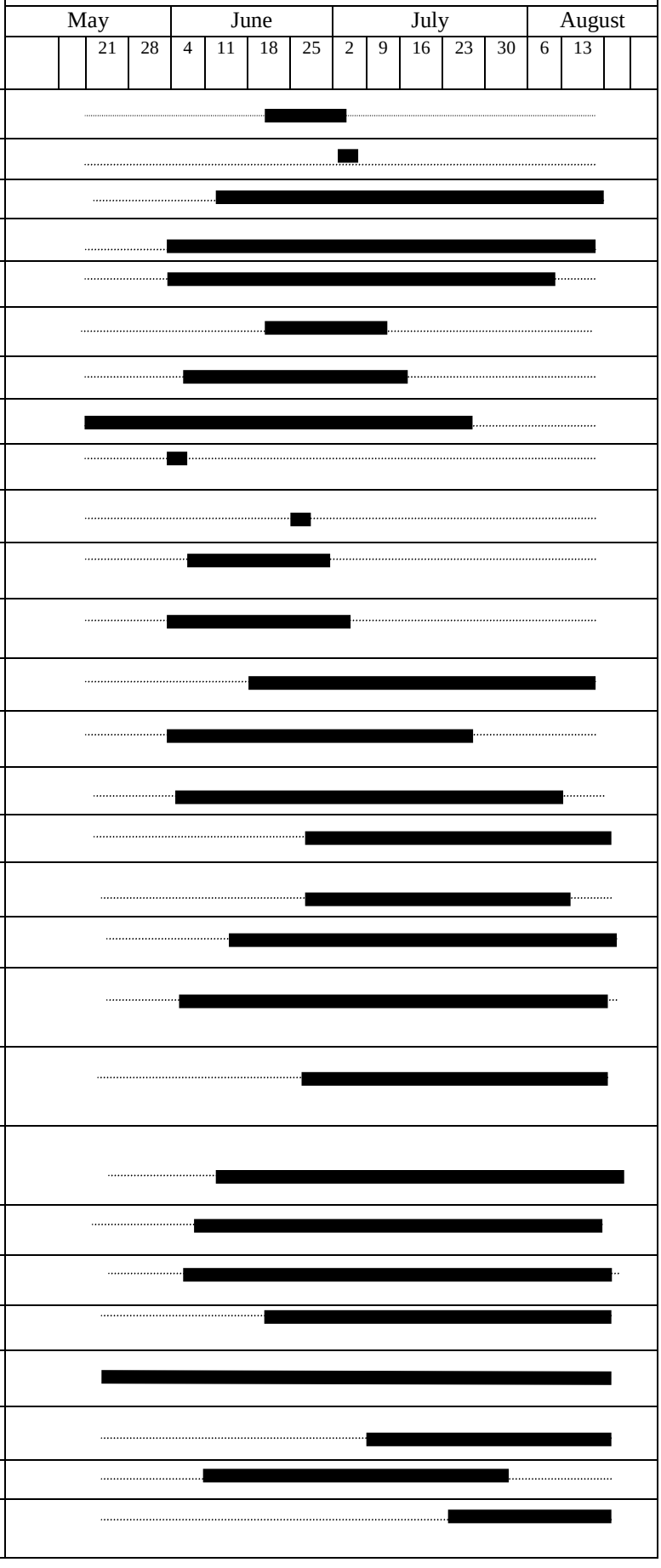
Appendix A. Field Data Sheets

TRAP SAMPLE DATA

ID/REF #	DATE/TIME SET	DATE/TIME RET.	GPS	STAGE/# OF INDIVIDUALS	H20 TEMP	AIR TEMP	WEATHER	NOTES

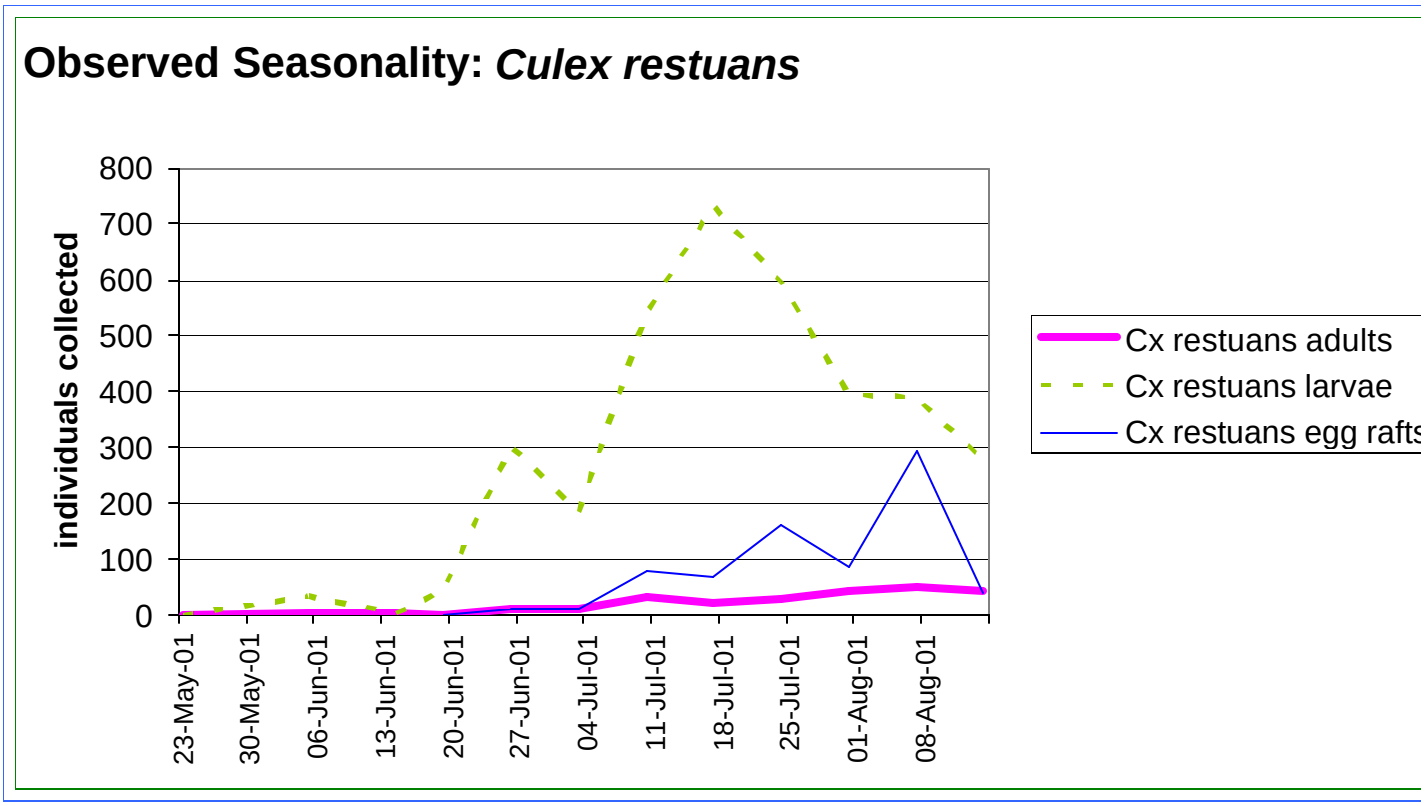
Appendix B. Portland Mosquito Population and Observed Seasonality

Species Collected In Portland (✣WNV Vector)	Total Number of Adults Collected	% of Adults Collected	Larva Collected	Observed Seasonality
Aedes abserratus	4	0.14		
Aedes atropalpus	1	0.03		
Aedes canadensis ✣	153	5.20	yes	
Aedes cantator ✣	417	14.30		
Aedes cinerus	162	5.60	yes	
Aedes communis	4	0.14		
Aedes excrucians	32	1.10		
Aedes fitchii	67	2.30		
Aedes intrudens	1	0.03		
Aedes japonicus ✣	1	0.03		
Aedes provocans	5	0.17		
Aedes punctor	13	0.45		
Aedes sollicitans ✣	23	0.79		
Aedes sticticus	5	0.17		
Aedes stimulans	365	12.50		
Aedes triseriatus ✣	117	4.00	yes	
Aedes trivittatus	18	0.62	yes	
Aedes vexans ✣	550	18.90	yes	
Anopheles punctipennis ✣	109	3.70	yes	
Anopheles quadrimaculatus	22	0.76	yes	
Coquillettidia perturbans ✣	179	6.10		
Culex pipiens ✣	340	11.70	yes	
Culex restuans ✣	251	8.60	yes	
Culex salinarius ✣	43	1.50	yes	
Culex territans	5	0.17	yes	
Culiseta melanura ✣	2	0.07		
Culiseta morsitans	2	0.07		
Uranotaenia sapphirina	25	0.86	yes	

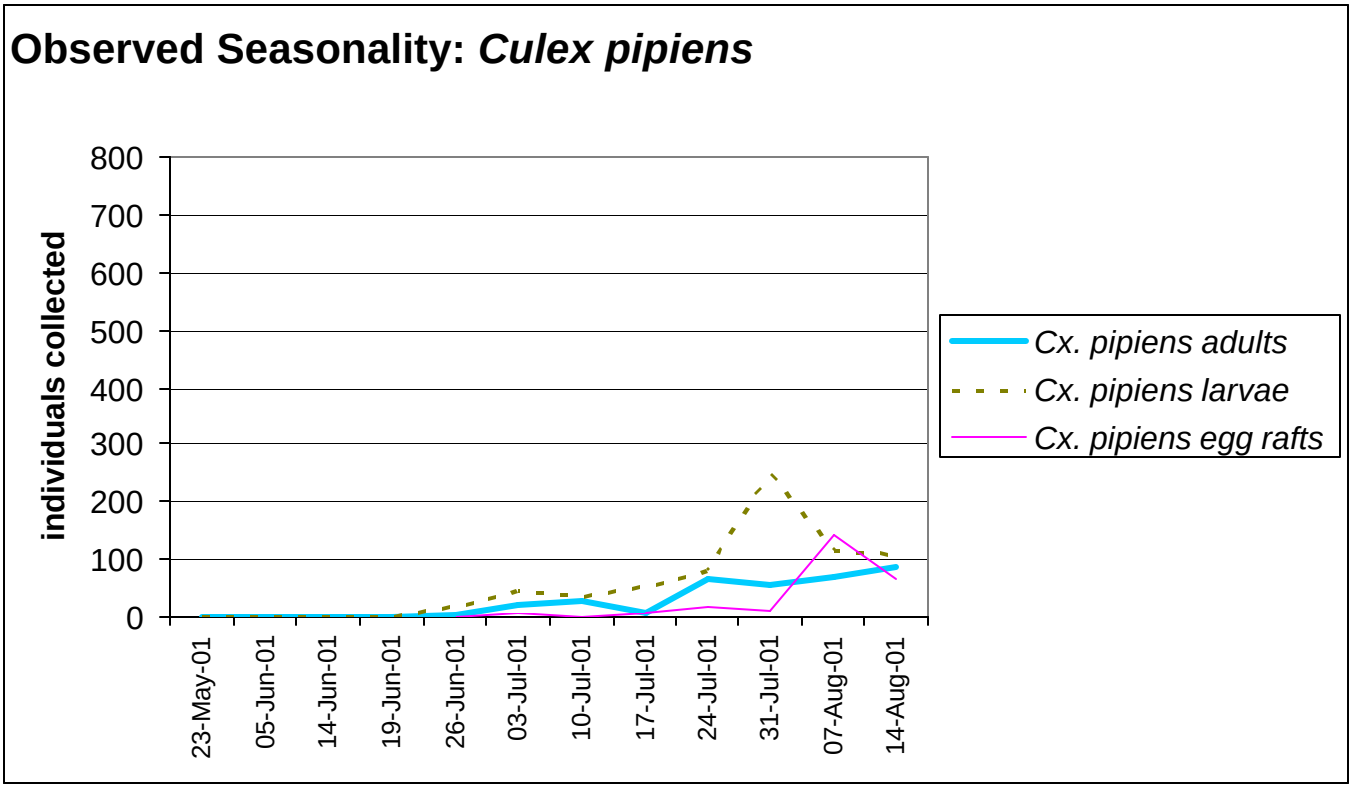


Appendix C. Portland Larval Sampling- 2001 Population and Seasonality

Larval Species	Larval Habitat	Human Biters	WNV Vector	Larval Observations										
				June				July				August		
				4	11	18	25	2	9	16	23	30	6	13
Aedes canadensis	wooded snowmelt pool	**Y**	**Y**	---										
Aedes cinerus	wooded snowmelt pool	**Y**		----										
Aedes triseriatus	shaded artificial /natural containers	**Y**	**Y**	----------										
Aedes trivittatus	grass/gravel puddles	**Y**	**Y**	----------										---
Aedes vexans	wooded snowmelt pool, grass/gravel puddles	**Y**	**Y**	----------									---	
Anopheles punctipennis	ponds	**Y**	**Y**	----------										
Anopheles quadrimaculatus	ponds	**Y**		---------										
Culex pipiens	shaded artificial /natural containers, shaded leaf/grass/gravel puddles and pools		**Y**	----------										
Culex restuans	shaded artificial /natural containers, shaded leaf/grass/gravel puddles and pools	**Y**	**Y**											
Culex salinarius	shaded artificial /natural containers, shaded leaf/grass/gravel puddles and pools	**Y**	**Y**	----------										----
Culex territans	ponds													
Uranotaenia sapphirina	shaded leaf/grass/gravel puddles and pools			----------										



DATE/TIME COLLECTED	*Cx restuans* adults	*Cx restuans* larvae	*Cx restuans* egg rafts
23-May-01	0	0	0
05-Jun-01	3	33	0
14-Jun-01	4	4	0
19-Jun-01	1	42	0
26-Jun-01	11	302	12
03-Jul-01	11	183	12
10-Jul-01	33	538	78
17-Jul-01	22	732	67
24-Jul-01	29	595	160
31-Jul-01	44	395	87
07-Aug-01	51	389	294
14-Aug-01	42	277	40



DATE/TIME COLLECTED	*Cx. pipiens* adults	*Cx. pipiens* larvae	*Cx. pipiens* egg rafts
23-May-01	0	0	0
05-Jun-01	0	0	0
14-Jun-01	1	0	0
19-Jun-01	0	0	0
26-Jun-01	4	16	0
03-Jul-01	21	45	7
10-Jul-01	29	34	0
17-Jul-01	8	51	6
24-Jul-01	65	78	16
31-Jul-01	54	245	12
07-Aug-01	70	114	142
14-Aug-01	88	108	65