Spring Species Composition and Sampling Techniques for Mosquitoes Collected Within the University of Florida's Natural Area Teaching Laboratory

Submitted by:
Chris Holderman, PhD Student
Dr. Phillip Kaufman, Associate Professor
Entomology and Nematology Department
Bldg. 970, Natural Area Dr. University of Florida, Gainesville, FL 32611-0620

Abstract

Mosquitoes are vectors of numerous human and animal pathogens including the causative agents of West Nile virus, yellow fever, dengue, filariasis and malaria. Understanding species diversity and the best trapping method is important to achieve control of pathogen vectors. Typically, mosquito sampling has been highly species or stage specific. Most researchers are interested in a specific population of mosquitoes or relative abundance to historical data, which are predominated by Centers for Disease Control and Prevention (CDC) style or similar suction traps. The techniques employed herein consisted of weekly 10-min. sampling events that compared two aspirator styles and sweep netting. This was followed by use of a carbon dioxide-baited suction trap. The goals of this project were to compare abundance and diversity captures using different active collection techniques, plot the seasonal trends in mosquito populations, and determine mosquito species presence for multiple trap sites in the Natural Area Teaching Laboratory.

The modified CDC backpack aspirator (Model 1412) collected the most (19) mosquitoes with 7 species recorded. A powered sweep aspirator (design from Ponlawat and Harrington 2005) generated an intermediate mosquito count with 8 individuals representing 6 species. Sweep netting preformed poorest of the active collection techniques, collecting only 2 species

and 2 individuals. The miniature CDC-style carbon dioxide-baited trap captured 21 species with 1,067 individuals collected over the 17-week collection period.

The seasonal phenology is presented for several species over the experimental time frame of the study is discussed. The winter of 2012-2013 was characterized by cooler than average weather extending through March, which may explain low mosquito collections during the middle of the study.

Introduction

Mosquitoes (Diptera: Culicidae) are a diverse family predominated by adult females that feed on the blood of vertebrates to obtain a protein source for vitellogenesis. Numerous mosquito species have been documented as pathogen vectors, causing diseases such as yellow fever, numerous encephalitides, dengue, filariasis, and malaria.

Mosquito field collections are typically conducted in a way that focuses on specific ages or biological stages. Centers for Disease Control and Prevention (CDC) style or similar light or carbon-dioxide traps attract mosquitoes that are seeking a blood meal. Additional traps exist that are attractive to ovipositing females. Within these two surveillance approaches, only a limited group of mosquitoes are ever sampled, inasmuch as some mosquitoes are only attracted to traps baited with specific CO_2 volumes while others will only approach ovitraps producing desired volatiles. However, most mosquitoes in the environment are neither seeking a host nor an oviposition site. This "resting" sub-population represents a majority within a mosquito population and in many respects, presents the best opportunity to sample diversity within the Culicidae. Therefore, the objective of this study was to sample the Natural Area Teaching Laboratory (NATL) to encumber the diversity of mosquitoes present between mid-winter and early spring through the comparison of the collection efficiency of resting mosquitoes by three

active techniques with a comparison to a standard CO_2-baited trap. By utilizing a multi-faceted sampling approach, this project generated a qualitative measure of species diversity in NATL.

Materials and Methods

The four collection techniques utilized included three active techniques: two aspirator models (large and small opening) and sweep netting, and a standard, passive technique utilizing a CO_2-baited host-seeking trap. The first aspirator was a modified CDC backpack aspirator model 1412 (John W. Hock Co., Gainesville, FL http://www.johnwhock.com/products/1412.htm). The second aspirator ("large aspirator") was a foliage aspirator (custom manufactured in-house, for description see Obenauer et al. 2010, design from Ponlawat and Harrington 2005). The third active collection technique was a sweep net (30 cm diameter) purchased from Bioquip (http://www.bioquip.com/search/DispProduct.asp?pid=7112NA). The fourth collection technique was a miniature CDC light trap model 512 with the light removed (http://www.johnwhock.com/products/512.htm). The trap utilized a cooler containing dry ice hung over the trap to dispense CO_2, and was used for comparative purposes to studies that did not utilize the active collection techniques.

Beginning January 7, 2013, the three active collection techniques were completed weekly in a randomized order in the public hammock area of NATL. Each collection technique used a 10 m transect, following the East Trail with a 5 m buffer between sampling regions. Sampling included the vegetation near the ground (< 1 m), leaf litter, rocks, tree trunks and other areas where adult mosquitoes are known to rest (Service 1976). These sampling techniques were followed by the placement and operation of a dry ice-baited CDC trap for 24 h near grid point H8. Samples were taken to the Veterinary Entomology Laboratory at the University of Florida

and identified using the keys of Darsie and Morris (2003). Sampling continued on a weekly basis until May 25, 2013, excluding weeks 8, 9, and 11.

The data generated were descriptive of the seasonality of when and which species of mosquitoes were active as spring advanced toward summer in NATL. Therefore, the mosquito counts were considered observational data and not analyzed. The comparative efficiency of each sampling technique is discussed below.

Results

The active collection techniques had limited success as collection tools when compared to the host-seeking trap. The least effective technique in both mosquito number and diversity was the sweep net, where only two specimens were recovered, *Aedes vexans* (Meigen) and *Culex quinquefasciatus* Say (Table 1). The large aspirator captured an intermediate number of mosquitoes, 8 in total, from *Aedes* (*Oc.*) *infirmatus* (Dyar and Knab), *Ae. vexans*, *Anopheles crucians* Wiedemann, *An. quadrimaculatus* Say, *Cx. quinquefasciatus*, and *Culex restuans* Theobald. The CDC backpack aspirator collected 19 mosquitoes including *Ae.* (*Oc.*) *infirmatus*, *Ae. vexans*, *Aedes* (*Oc.*) *mitchellae* (Dyar), *Culex nigripalpus* Theobald, *Cx. quinquefasciatus*, *Cx. restuans*, and *Culex salinarius* Coquillet. Male mosquitoes were collected with the females during aspirations, but were not identified or reported. The miniature CDC style CO_2-baited trap captured 21 species with 1,067 individuals collected over 17 weeks, as shown in Table 2.

Overall, the diversity of the NATL mosquito populations during the sampling period was compromised of 22 species. For 12 of these species, 10 or fewer individuals were collected. Three species compromised 70% of mosquitoes collected; *An. crucians*, *An. quadrimaculatus*, *Ae.* (*Oc.*) *infirmatus*. The miniature CDC style CO_2-baited trap captured 17 species that were not present in other collection techniques. Sweep netting and the large aspirator did not yield any

not previously collected species. Whereas the CDC backpack aspirator collected one species (*Ae.* (*Oc.*) *mitchellae*) not captured in the miniature CDC style CO_2-baited traps.

Table 1. Comparison of three active mosquito collection techniques conducted for 10 min periods over a 10 m area in the Natural Area Teaching Laboratory over a 17-week period.

Week	Mosquito Species	Number Captured
Sweep Netting		
1	*Aedes vexans*	1
5	*Culex quinquefasciatus*	1
Large Aspirator		
1	*Ae. vexans*	1
2	*Aedes* (*Ochlerotatus*) *infirmatus*	1
2	*Anopheles quadrimaculatus*	1
2	*An. crucians*	1
3	*Cx. restuans*	1
3	*Ae. vexans*	1
7	*Cx. quinquefasciatus*	1
17	*Cx. quinquefasciatus*	1
CDC Backpack		
1	*Ae.*(*Oc.*) *infirmatus*	1
2	*Ae. vexans*	1
3	*Cx. nigripalpus*	2
4	*Cx. salinarius*	1
4	*Ae.*(*Oc.*) *infirmatus*	1
4	*Cx. nigripalpus*	9
7	*Cx. quinquefasciatus*	1
10	*Cx. quinquefasciatus*	1
12	*Ae.* (*Oc.*) *mitchellae*	1
17	*Ae.* (*Oc.*) *infirmatus*	1

Week denotes calendar week of 2013, week 1 = Jan. 7, week 17 = Apr. 22. Collections were not completed in weeks 8, 9, 11

Table 2. Mosquito species and number of females captured in a CO_2-baited miniature CDC light trap operated one night per week from 07 January 2013 through 22 April 2013.

	Calendar Week of the Year (2013)														
Mosquito Species	1	2	3	4	5	6	7	10	12	13	14	15	16	17	Total
Anopheles crucians	16	-	15	6	4	-	24	1	53	6	14	16	11	3	169
An. quadrimaculatus	10	1	14	5	14	14	30	12	4	3	1	1	-	2	111
Aedes albopictus	-	-	1	-	-	-	-	-	-	-	-	-	1	1	3
Ae. triseriatus	-	-	-	-	-	-	-	-	-	-	-	6	4	4	14
Ae. vexans	11	-	18	2	2	1	2	3	2	1	8	1	2	3	56
Ae. (*Oc.*) *atlanticus*	-	-	-	-	-	-	-	1	-	-	-	-	-	2	3
Ae. (*Oc.*) *C. canadensis*	-	-	3	-	-	-	-	-	14	-	-	-	-	-	17
Ae. (*Oc.*) *infirmatus*	111	-	7	2	2	-	1	-	16	37	108	95	82	27	488
Ae. (*Oc.*) *thibaulti*	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Culiseta melanura	2	-	3	-	-	-	-	-	-	-	-	1	12	-	18
Culex coronator	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Cx. erraticus	9	-	-	-	-	-	-	-	-	-	-	-	1	-	10
Cx. nigripalpus	3	-	-	-	1	-	1	-	-	-	-	-	-	-	5
Cx. quinquefasciatus	-	2	-	4	2	1	1	-	-	-	-	2	4	1	17
Cx. restuans	1	-	4	-	-	-	-	-	-	-	-	-	-	-	5
Cx. salinarius	14	3	3	4	12	1	3	2	-	-	-	2	12	8	64
Cx. tarsalis	8	-	1	-	-	-	-	-	-	-	-	-	-	-	9
Cq. pertrubans	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Psorophora ciliata	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Ps. howardii	-	-	-	-	-	-	-	-	2	-	-	-	-	-	2
Ps. ferox	-	-	-	-	-	-	-	-	3	1	19	18	20	11	72
Total	185	6	69	23	37	17	63	19	94	48	150	142	150	64	1,067

Oc. = Ochlerotatus.

Collections were not completed in weeks 8, 9, 11

Discussion

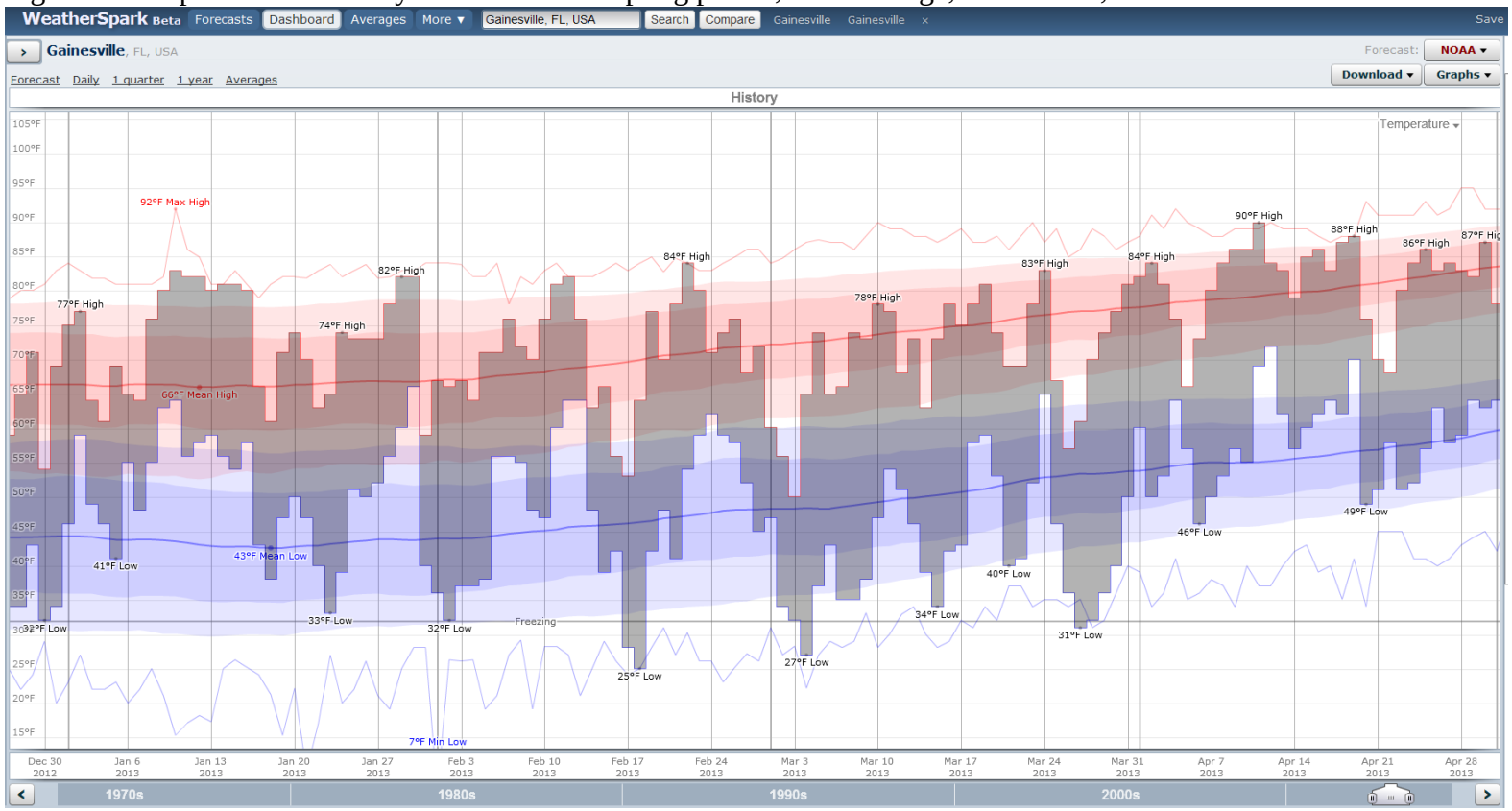
During the study period the temperatures in the NATL were colder than average and likely explains in part why the number of collected mosquitoes was low (Fig. 1). Future studies should consider the varied topography and foliage cover of NATL when choosing a collection technique, as this could influence the species diversity obtained with aspirator sampling. The two aspirators are inherently different with the large aspirator surrounding vegetation and pulling mosquitoes from the foliage. Whereas, the CDC backpack aspirator utilizes a focused suction with a smaller orifice and must be specifically directed by the operator at targeted sites, such as with mosquito removal from tree holes and other confined spaces. The vegetation in NATL is not manicured as one would find in a residential area and may have provided too many resting sites to effectively utilize the large foliage aspirator in the confined time allotted through the experimental protocol. Use of sweep netting as a mosquito sampling technique should be avoided, as it was ineffective at collecting mosquitoes from the habitat.

The phenology of species composition may be confounded by not including collections prior to the January initiation. Overall, mosquito numbers remained low for several weeks into the study, but our results do document mosquito presence and host seeking behavior during the winter when daylengths are the shortest. The three most numerous mosquito species (*Ae. infirmatus*, *An. crucians*, and *An. quadrimaculatus*) from the CDC trap represents 70% of the collected species over the study period. However, dominance of these three species was not represented among the active collection techniques. Overall, the active techniques were either ineffective, may need greater mosquito populations to reflect patterns from the baited-trap or suggest that *Ae. infirmatus*, *An. crucians* and *An. quadrimaculatus* were not using resting sites within NATL and moved toward the CO_2-baited traps from an outlying area.

Table 3. List of mosquito species captured in the Natural Area Teaching Laboratory from 07 January 2013 through 22 April 2013

Mosquito species
Anopheles crucians
Anopheles quadrimaculatus
Aedes albopictus
Aedes triseriatus
Aedes vexans
Aedes (*Ochlerotatus*) *atlanticus*
Aedes (*Ochlerotatus*) *canadensis canadensis*
Aedes (*Ochlerotatus*) *infirmatus*
Aedes (*Ochlerotatus*) *mitchellae*
Aedes (*Ochlerotatus*) *thibaulti*
Culiseta melanura
Culex coronator
Culex erraticus
Culex nigripalpus
Culex quinquefasciatus
Culex restuans
Culex salinarius
Culex tarsalis
Coquillettidia pertrubans
Psorophora ciliata
Psorophora howardii
Psorophora ferox

Figure 1. Tempature recorded by NOAA over sampling peroid, with average, maximums, and minimiums.



Data retrieved from http://weatherspark.com

References

Darsie Jr., RF and RA Ward (2004) *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. Gainesville, FL, Univ. of Florida Press. pp. 383

Obenauer, PJ, PE Kaufman, DL Kline, and SA Allan. 2010. Detection of and monitoring for *Aedes albopictus* (Diptera: Culicidae) in suburban and sylvatic habitats in North Central Florida using four sampling techniques. Environ. Entomol. 39: 1608-1616.

Ponlawat, A, and LC Harrington. 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. J. Med. Entomol. 42: 844-849.

Service, M. W. (1976) *Mosquito ecology : field sampling methods*. Wiley, New York.

Weatherspark beta.
http://weatherspark.com/#!dashboard;q=Gainesville%2C%20Florida%2C%20United%20States