Ovary Dissections for Parity Determination of Field-Collected *Coquillettidia perturbans* Mosquitoes in Central Massachusetts

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ABSTRACT

The aim of this study was to determine the changes in the age structure of the *Coquillettidia perturbans* mosquito population in Central Massachusetts during the middle of the summer in 2013. This project was carried out in conjunction with the retention pond surveillance for *Coquillettidia perturbans*, which has been carried out for several years by the Field Biologist Frank Cornine at the CMMCP. This mosquito species is of particular interest because it is a known vector of the Eastern Equine Encephalitis (EEE) virus in Massachusetts. Conclusions about the changes in the age structure of the population are limited due to the abbreviated time span of this study. This study provides the groundwork for future similar studies that may include the entire mosquito season.

INTRODUCTION

The 2012 Arbovirus Surveillance Summary for Massachusetts reported that 3.9% of the mosquito samples tested were positive for Eastern Equine Encephalitus (EEE). There were seven human cases of EEE in Massachusetts in 2012 and all but one developed as meningoencephalitis (MDPH, 2012). The enzootic vector species for the EEE virus in Massachusetts is *Culiseta melanura*, but *Coquillettidia perturbans,* along with several other species, have tested positive for EEE and are suggested bridge vectors. An enzootic vector maintains the viral cycle in animals, while a bridge vector transmits the virus to a dead-end host such as a human or a horse (CDC, 2013). *Coquillettidia perturbans* is known to blood feed on birds, humans, and other mammals. This trait of biting both humans and birds along with its abundance during the summer makes it a potential bridge vector for the EEE virus (Armstrong & Andreadis, 2010).

It is not known definitively whether there is a single peak of emergence of the *Coquillettidia perturbans* population every summer in Central Massachusetts or several peaks. Previous years of population abundance data have shown that typically there is a peak in the population in mid-June and *Coquillettidia perturbans* remains common until mid-August (CMMCP, 2013). This pattern of one peak of emergence has been found in other northern states such as Ohio (Nasci et al., 1996) and Michigan (Olds et al., 1989). This single peak of emergence in northern populations is referred to as “univoltine”, while two

peaks is called “bivoltine” and has been observed in southern regions (Lounibos & Escher 1983). A previous study in Ohio found a largely univoltine pattern using population size, parity structure, and wing length of *Coquillettidia perturbans* mosquitoes (Nasci et al., 1996). However, the percentage of parous mosquitoes dropped at the end of August, indicating a smaller, second adult emergence of *C. perturbans* (Nasci et al., 1996). To discover if this type of pattern might also occur in Massachusetts, it is necessary to collect information on parity.

Previous years of surveillance for *Coquillettidia perturbans* in Central Massachusetts have indicated a single peak of emergence at the end of June and early July. The district 3 and 4 graphs from 2013 are included because these are the districts where the mosquitoes for this study were trapped. (Figures 1 & 2). Figure 1 shows a univoltine peak at EPI week 26 for the 7-year average. The 2013 data has a later peak of emergence. Both of these peaks are within EPI weeks 26-30, which is when data was collected on parity in the summer of 2013. In the 2013 data there is a notable second peak of emergence in week 35. Figure 2 has a plateau of emergence from EPI weeks 26-30 for the average data as well as the 2013 data.

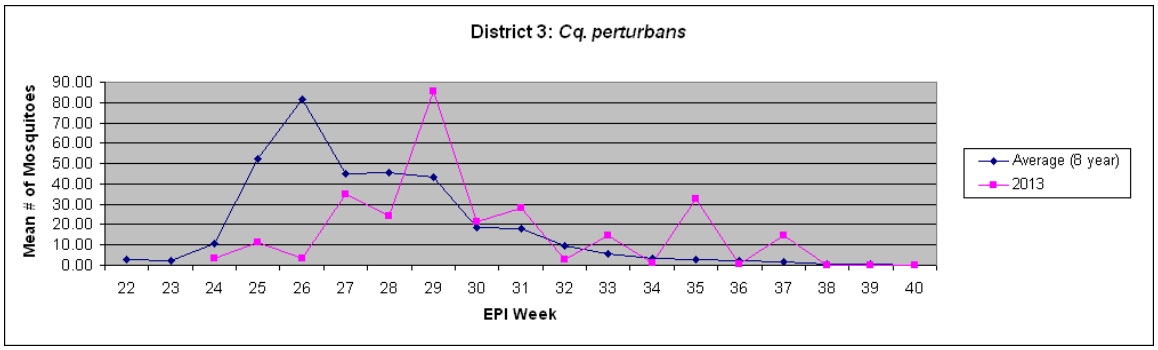

Figure 1. Central Massachusetts Mosquito Control Project 2013 District 3 graph of the *Coquillettidia perturbans* population numbers for the 2013 season compared to the 7-year averages. (Cornine, 2013)

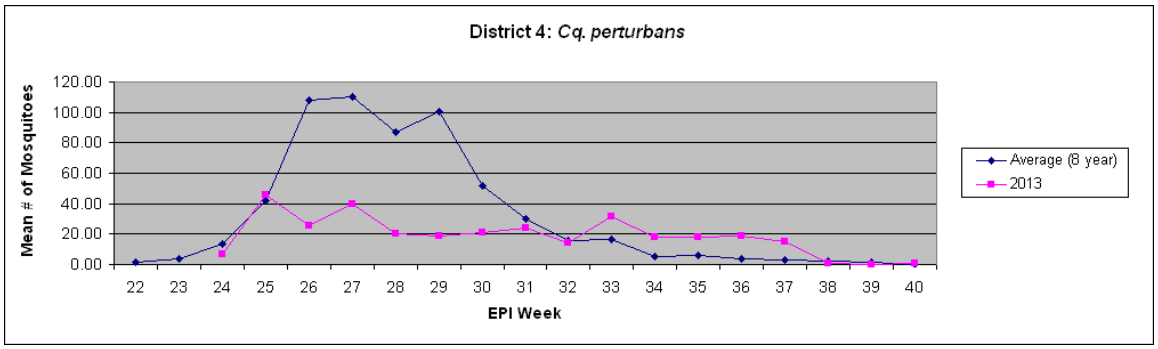

Figure 2. Central Massachusetts Mosquito Control Project 2013 District 4 graph of the *Coquillettidia perturbans* population numbers for the 2013 season compared to the 7-year averages. (Cornine, 2013)

The parity status of an adult female mosquito indicates whether the female has oviposited during her lifetime or not. A female is nulliparous either if she is newly emerged or if she was not able to find a blood meal. A female is parous if she has previously bloodfed and oviposited (Service, 2012). A parous mosquito is more likely to be carrying a virus because it has bloodfed at least once on a potentially infected host.

The parity status of trapped *Coquillettidia perturbans* mosquitoes in Central Massachusetts can be used to estimate the age structure of the population and confirm whether there is one peak of emergence or several. The determination of parity is relevant to the surveillance for virus-carrying mosquitoes. Younger females that have not yet bloodfed do not carry the EEE virus, while older females are more likely to be infected because they have blood fed at least once. Older females also tend to feed more often and on a greater variety of hosts, increasing the chance of transmitting a virus from a bird to a human. Residents should be more concerned about having an older population of mosquitoes on their property than a few newly emerged mosquitos.

The method used to determine parity in this study was described by Detinova (1962) and is called the ovary tracheation method. The presence of coiled tracheole skeins in a dried ovary indicates a nulliparous mosquito and uncoiled skeins indicates a parous mosquito. This is based on the fact that the ovary expands during egg maturation and the tracheoles permanently uncoil (Detinova, 1945; Hoeck PA, et al 2003). This method of age grading of mosquitoes will contribute to the data on the changes in the *Coquillettidia perturbans* population over the course of the summer in Central Massachusetts.

This study aims to answer two research questions: What are the patterns of the *Coquillettidia perturbans* population age structure in the Central Mass region? Is there one peak of emergence and then the population slowly ages, or are there multiple peaks of emergence throughout the summer?

Methods

In order to determine the age structure of the *Coquillettidia perturbans* population in Districts 3 and 4 of the CMMCP coverage area, several surveillance sites were chosen next to retention ponds. They were located on West Union Street in Ashland, Madden Ave in Milford, Smith Parkway in Westborough, and Centech Boulevard in Shrewsbury. These sites did not receive any insecticide application during the summer and are not located in residential areas. The CO_2 baited CDC traps were run overnight and set out and collected 2 times per week. A device (HOBO U23 Pro RH/Temp Logger; Onset Computer Corporation, Pocasset, MA) was attached to each trap to monitor the temperature and humidity levels at the trap site.

The collected mosquitoes were placed in a cooler with a cold pack until they were brought back to the CMMCP building. When the mosquitoes were returned to the CMMCP office, they were knocked down with Trimethylene and transferred to a collection cup with a moist paper towel. Then they were kept at 4^0 C in the fridge or on wet ice until they were identified. After separating out the species that are focused on in this study (*Coquillettidia perturbans*), the mosquitoes were either dissected right away or kept in mosquito saline (protocol from Dr Richard Pollack) at 4^0C until the next day for dissection. Keeping the mosquitoes cold and moist reduces the drying out of the specimen and therefore improves the dissection efficiency. They should not be left in saline for more than 4 days for maximum tissue quality.

The ovaries were dissected from 24 *C. perturbans* mosquitoes for each surveillance site collection and dried at room temperature on a labeled slide. The parity of each set of dried ovaries was determined using the tracheal skeins method described by Detinova (1962). This method has been found to be accurate and relatively simple to carry out (Hugo, et al., 2008). The presence of coiled tracheole skeins indicates a nulliparous mosquito and uncoiled skeins indicate a parous mosquito. The slides with dried ovaries can be stored in a clean slide box and at room temperature indefinitely.

Results

The results of the parity determination for the *Coquillettidia perturbans* mosquitoes collected from EPI week 26-30 (last week of June – 4th week of July) are shown in Figures 3-6. The number of undetermined ovaries was subtracted from the total number of dissected and dried ovaries from that trap, and then the percent nulliparous was calculated out of that adjusted total for that trap.

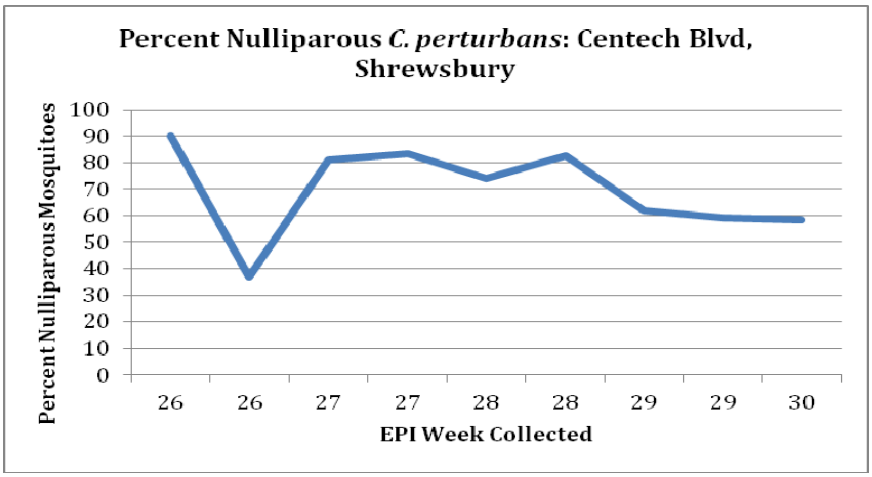

Figure 3. District 3 site on Centech Boulevard, Shrewsbury. The percent of nulliparous mosquitoes out of the total number dissected which were identified as either nulliparous or parous. This includes EPI weeks 26-30 (June 25 – July 23, 2013).

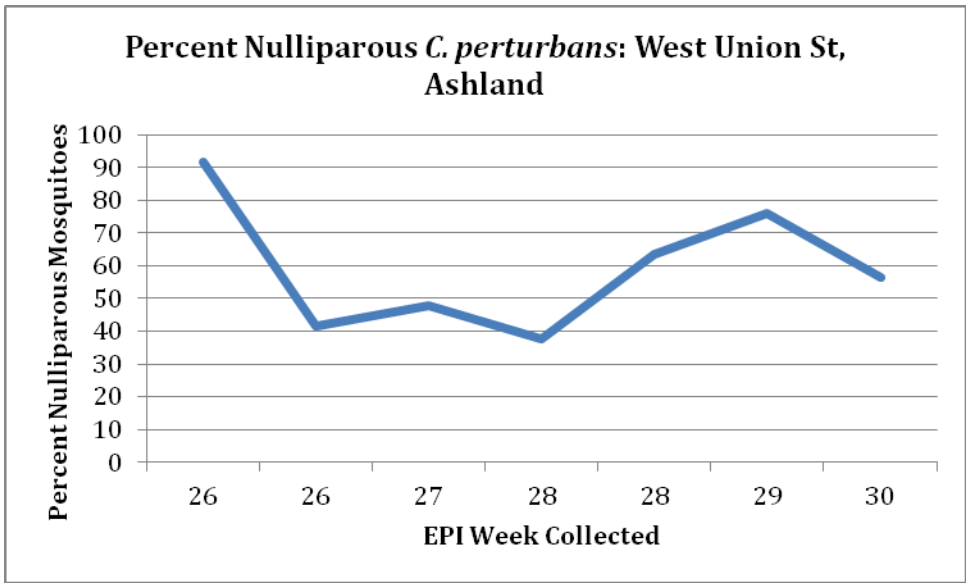

Figure 4. District 4 site on West Union Street, Ashland. The percent of nulliparous mosquitoes out of the total number dissected which were identified as either nulliparous or parous. This includes EPI weeks 26-30 (June 25 – July 23, 2013).

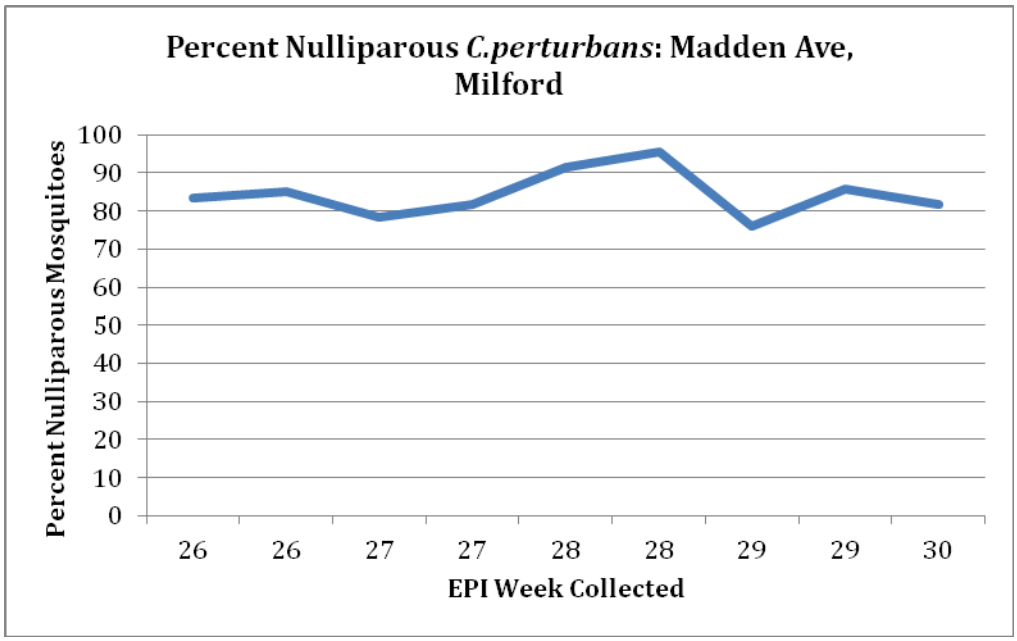

Figure 5. District 4 site on Madden Ave, Milford. The percent of nulliparous mosquitoes out of the total number dissected which were identified as either nulliparous or parous. This includes EPI weeks 26-30 (June 25 – July 23, 2013).

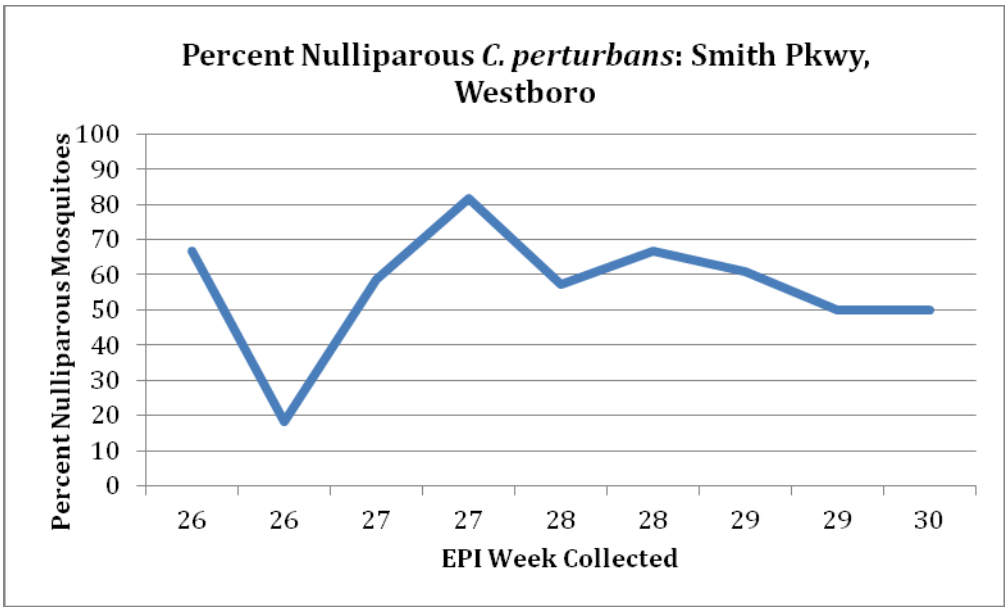

Figure 6. District 4 site on Smith Parkway, Westborough. The percent of nulliparous mosquitoes out of the total number dissected which were identified as either nulliparous or parous. This includes EPI weeks 26-30 (June 25 – July 23, 2013).

Except for the drop during the second collection in EPI week 26 and a small drop on West Union Street, the percent nulliparous remained over 50% when this data was collected. EPI week 26 had an emergence event (high percent nulliparous) just prior to the collection early in the week, and then for three of the sites (Figures 3, 4, 6) the percent nulliparous decreased before the collection several days later in the same EPI week. Centech Boulevard (Figure 3) had an average of 69.66% nulliparous *Coquillettidia perturbans*. West Union Street (Figure 4) had an average of 59.29% nulliparous *C. perturbans.* The data and graph from West Union Street exclude collection two from week 27 due to only two *C. perturbans* collected due to a trapping problem. Also collection two from week 29 on West Union Street was excluded due to a problem with the specimens drying out and failed dissections. Madden Avenue (Figure 5) had an average of 84.38% nulliparous *C. perturbans*. Smith Parkway (Figure 6) had an average of 56.69% nulliparous *C. perturbans.*

Discussion

This study demonstrated the feasibility of determination of parity from select populations at these field sites. The graphs shown in the results section give us parity information about a portion of the 2013 mosquito season in Central Massachusetts. There were two trapping days in each of the EPI weeks 26-29. A drop in the percent of nulliparous mosquitoes can be seen in three out of the four sites (Figures 3, 4, 6) during EPI week 26. The drop in nulliparous mosquitoes between the two collections in EPI week 26 indicates that many of the newly emerged mosquitoes from the beginning of the week were able to blood feed and lay eggs before the second collection that week. The variability within one EPI week of

collections indicates that it is advantageous to have two collections in one week to increase reliability in each EPI week's average data on parity.

Over 50% of the *Coquillettidia perturbans* mosquitoes were nulliparous for the majority of the collections. This could indicate that the peak in the *C. perturbans* population is not due to one week of mass emergence, but several weeks of large numbers of emerging mosquitoes. This could explain the plateau-like peak in the 7-year average data in the District 4 graph (Figure 2). The study was not continued into the period of the season in which we would expect to see a dramatic decline in the population and therefore an increase in older, parous, mosquitoes.

The Central Massachusetts Mosquito Control Project (CMMCP) uses both adulticides and larvicides as part of an integrated pest control approach to reduce mosquito populations and subsequently arbovirus transmission. The CMMCP has not used an age grading method to assess the age structure of the mosquito population in the past. This study contributed to the efficiency research already taking place at CMMCP and laid the groundwork for future studies using parity.

If a future study is done which comprises the entire season, conclusions could be drawn about the age structure patterns of *Coquillettidia perturbans* mosquitoes in Central Massachusetts. If there are clear points in the season during which larva are the most abundant, maybe larviciding for this species could be done then every year. However it is difficult to use larvicidal pesticides against this species because the C. *perturbans* larvae use their siphon to pierce cattails and access air in a way that circumvents most larvicide products. If there is a clear time in the season when the majority of the mosquitoes are parous and therefore more likely to carry EEE virus, maybe adulticiding would be most effective at that time in the season. Future parity determination studies would be useful in expanding the understanding of mosquito populations in Massachusetts and how best to control them and protect the public from arboviruses.

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