Overwintering *Cs. melanural* EEE Vertical Transmission Study 2012

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During the 2012 mosquito season there was increased EEE activity throughout Massachusetts. Within the CMMCP EEE virus was isolated ten times from mosquito collections taken from five towns. Two of these five towns had multiple isolations of EEE. The town of Westborough produced five positive isolations and the town of Berlin produced two isolations. A human fatality due to EEE infection was reported from Westborough as well.

In November of that year Dr. Andreadis of the Connecticut Agricultural Experiment Station asked Massachusetts mosquito control projects to provide him with Cu*liseta melanura* larvae from locations where EEE isolations were made during the recently concluded season. Dr. Andreadis theorized that due to the recent record breaking EEE activity in Massachusetts there might be a better opportunity to find evidence of vertical transmission of EEE within the *Culiseta melanura* population than had ever existed before. Therefore the CMMCP Staff Entomologist began to make collections of *Cs. melanura* larvae.

The effort was centered on two locations where multiple EEE isolations had been made during 2012. The first of these locations was a Red Maple swamp in Westborough. The swamp lies between Summer Street Extension, Lyman Street and Boston Turnpike (Rt. 9). The second location was a Red Maple swamp located northeast of Sawyer Hill Road and Route 62 in Berlin

Multiple transects on numerous occasions were made of both swamps. Each time the cryptic larval habitats associated with *Cs. melanura* were sampled with a modified boat bilge pump. Water samples suctioned from a habitat were placed in a flat enamel pan and than observed for larval activity. Larvae observed in the water sample were suctioned from the pan with a pipette and transferred to a glass vial. On other occasions water samples were suctioned from the habitat and transferred to a four gallon plastic bucket. Excess water was regularly tipped off the bucket thereby concentrating the samples. The contents of the bucket were than brought back to the laboratory and poured into two 2 gallon plastic tubs. The detritus was allowed to settle overnight. The next day the water in the tubs was observed for mosquito larvae activity. Larvae were captured with a pipette and transferred to a rearing chamber.

On some collecting expeditions water samples were strained through a fine sieve thereby reducing the amount of water that had to be observed or transferred back to the laboratory. Between crypts the sieve was carried in a 4 gallon bucket that contained

enough water to keep the contents trapped in the sieve wet. Upon returning to the laboratory the contents of the sieve were placed in a 1 gallon plastic pretzel container that had had holes drilled in the collar. The cap of the pretzel container had also been modified by removing the flat top of the cap so that it was more like a ring. A plastic funnel was also modified by drilling holes in the sides where it was widest. The wide end of the funnel was than glued to the cap of the pretzel container. The modified cap with the funnel attached was than screwed onto the container. The pretzel container was than placed in a five gallon plastic bucket. The bucket was slowly filled with water from a garden hose until the water surface was one inch above the tip of the funnel. Soon after the pretzel container was completely immersed in clean water mosquito larvae made their way from the turbid crypt water contained in the pretzel container through the tip of the cone and into the cleaner water in the bucket. There the larvae were more easily suctioned with a pipette.

At Dr. Andreadis' request the larvae were separated by origin and by instar. Once categorized by instar the larvae were placed in glass vials and held in a – 80 Centigrade freezer until arrangements could be made for their transfer to Dr. Andreadis' laboratory. The results of the survey are included below.

			CMMCP , Northborough, MA			
			EEEv Larvae Project 2012			
Tube #	**Date**	**Site**	**Species**	**Instar**	**Amount**	
T 1	**XI - 12**	**Berlin**	***Cs. melanura***	**2nd**	**6**	
T 2	**XI - 12**	**Berlin**	***Cs. melanura***	**3rd**	**1**	
T 3	**XI - 12**	**Westborough**	***Cs. melanura***	**2nd**	**47**	
T 4	**XI - 12**	**Westborough**	***Cs. melanura***	**3rd**	**50**	
T 5	**XI - 12**	**Westborough**	***Cs. melanura***	**3rd**	**10**	
T 6	**XI - 12**	**Westborough**	***Cs. melanura***	**4th**	**50**	
T 7	**XI - 12**	**Westborough**	***Cs. melanura***	**4th**	**39**	
					203	

The larval collecting season came to an end when temperatures dropped low enough to freeze the water in the swamps thereby making it impossible to sample crypts.

Two other Massachusetts' mosquito control projects participated in this study. Their larval samples were delivered to the CMMCP office to await retrieval by CAES personnel. Dr. Andreadis did say that 3000 – 10,000 Cs. melanura larvae would be needed to make a proper sample for his research into vertical transmission of EEE in Culiseta melanura. The combined collections of the mosquito control projects did not arise to that number. Dr. Andreadis has not contacted anyone that I know of to say what results if any were produced as a result of this research.