

# Overwintering mosquitoes in Illinois stormwater tunnels & caves

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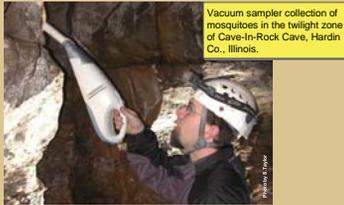
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*Culex pipiens pipiens*  
Auctioneer Cave  
Monroe Co., Illinois

## Objective

Mosquitoes have long been known to over-winter in caves in Illinois and elsewhere in the United States (Banta 1907, Hinman & Hurlbut 1940, Ross & Horsfall 1965). Our goal was to make species-level comparisons between mosquitoes wintering in urban stormwater tunnels (McClellan 2000) with those in rural cave settings, assessing the later for the presence of WNV.

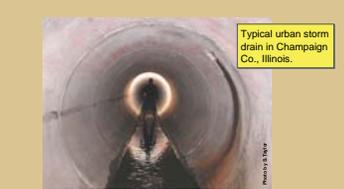


Vacuum sampler collection of mosquitoes in the twilight zone of Cave-In-Rock Cave, Hardin Co., Illinois.

## Methods

Overwintering mosquitoes were collected in the entrance and twilight zones of caves. Caves in southern Illinois were sampled and compared with previous stormwater tunnel data from central Illinois (McClellan 2000). Cave collections employed a battery powered vacuum sampler, which allowed qualitative sampling of adult mosquitoes from walls and ceilings of caves. Mosquitoes were transported back to the laboratory in a humidified atmosphere cooled with ice, and collections were maintained live in screened enclosures in a walk-in incubator, with each cage having access to honey and water.

Live specimens were chilled and identified under a stereomicroscope. In cases where identification of *Culex* in the *pipiens* complex could not be done, the mosquitoes were ground and DNA extracted for amplification of different portions of diagnostic genes (Sanogo et al. 2008). For WNV detection, a portion of the mosquito body that had been excised was RNA extracted and tested with primer sets for WNV-RNA by RT-PCR TaqMan (Lampman et al. 2006).



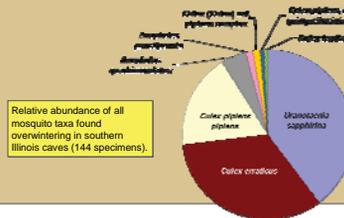
Typical urban storm drain in Champaign Co., Illinois.

## Results

Six caves in southern Illinois were sampled between 2 February and 7 March, 2008, when mosquitoes were in hibernation. Mosquitoes were recovered from all caves, with an average of 2.83 taxa/cave. However, two caves contained five taxa, one contained four taxa, and three caves contained one taxon.

Two species are dominant in the cave samples: *Uranotaenia sapphirina* (Osten Sacken) (57 specimens) and *Culex erraticus* (Dyar and Knab) (48 specimens). *Culex pipiens* Linnaeus was represented by 26 specimens and *Culex territans* Walker by one specimen. Two specimens belong to the subgenus *Culex* but are not members of the *pipiens* complex and did not amplify for any known member of the subgenus in our area in the molecular identification procedure. One specimen is a cross between the northern and southern house mosquitoes (*Cx. pipiens* and *Culex quinquefasciatus* Say), which is increasingly expected towards the south. *Anopheles quadrimaculatus* (Say) *sensu lato* and *Anopheles punctipennis* (Say) (7 and 2 specimens, respectively), make up the remainder of the material collected.

None of the cave samples were positive for West Nile virus.



Relative abundance of all mosquito taxa found overwintering in southern Illinois caves (144 specimens).

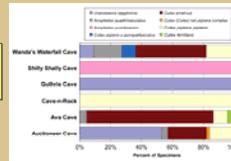
## Discussion

We collected mosquitoes from six caves and found *Cx. pipiens*, *Cx. erraticus*, *Cx. territans*, *A. punctipennis*, *A. quadrimaculatus* and *U. sapphirina*. One *Culex* (*Culex*) specimen, not in the *pipiens* complex, could not be identified to species level, and on specimen is intermediate between *Cx. pipiens* and *Cx. quinquefasciatus* based on molecular analysis. Individual caves harbored from one to five taxa. Studies in Illinois, Indiana, and Tennessee over a century earlier tended to find low diversity of mosquitoes in individual caves. *A. punctipennis* was usually more common than *A. quadrimaculatus*, the midwestern primary vector of malaria before its eradication in the US. All of these species have been associated with WNV in some state and some southern areas *Cx. erraticus*, which we found in abundance, is potentially the major vector of WNV (Cupp et al. 2007).

The bionomics of the species in the caves provide some insight into their ecology. For example, *U. sapphirina* feeds on amphibians (Carpenter and LaCasse, 1955), and caves where this species was recorded are known to contain amphibian taxa as well as having nearby habitat that would harbor amphibians during the spring summer months. *Culex erraticus* has a broad feeding range (mammals, birds, and amphibians) and has been shown to be positive for WNV (Cupp et al. 2007). *Anopheles quadrimaculatus* s.l., which was the primary vector of malaria in the midwest - including Illinois - during the mid-1800s, tends to feed large mammals (Levine et al. 2004).

In an earlier study of Illinois' cave fauna, Peck and Lewis (1978) recorded *A. punctipennis* and *Cx. pipiens* from several Illinois caves, noting that both commonly overwinter in caves. Webb et al. (1994) reported mosquitoes occurring primarily in the twilight (66.7%) and entrance (28.9%) zones of Illinois caves, with few (4.4%) in the dark zone. *Culex pipiens* is the primary vector of St. Louis encephalitis virus and WNV in Illinois, causing thousands of cases. The feeding behavior of this species is often described as primarily avian, but recent studies suggest this may be influenced by host availability. In Missouri, Barnes (2004) found Ozark caves contained primarily four species: *A. punctipennis*, *Cx. erraticus*, *Cx. pipiens* or *Cx. quinquefasciatus*, and *U. sapphirina*, and in Indiana, Shroyer & Siverly (1973) recorded *Cx. pipiens* overwintering in caves.

Proportion of overwintering mosquitoes belonging to each taxon for six southern Illinois caves.



## Conclusions

The overwintering, facultatively troglonectic culicid fauna of caves in southern Illinois is similar to that of southern Missouri and northern Arkansas and Indiana. The fauna of stormwater tunnels in Champaign Co., Illinois differ somewhat from the cave fauna, especially in the absence of *U. sapphirina*. In both habitats, overwintering culicid faunas are comprised of species known to be WNV vectors, and some have been associated with malaria transmission.

Humans create hibernacula for some vector species by building vast underground stormwater systems that mimic naturally occurring, interconnected underground systems (caves). However, Illinois stormwater tunnels are often dominated by *Cx. pipiens* adults, unlike the caves. These differences can be explained the surrounding habitat - *Cx. pipiens* tends to use anthropogenic larval breeding sites such as artificial containers, domesticated animal sewage lagoons, and catchbasins, and is less abundant in rural and natural areas, where *Cx. restuans* tends to be more prevalent. The latter species was not found in any of the caves surveyed.



*Culex pipiens pipiens*  
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## Introduction

Mosquitoes in temperate areas of North America typically survive the winter in either a facultative reproductive diapause in the adult stage (*Culex*, *Culiseta*, *Uranotaenia* and *Anopheles* spp.) or as dormant, desiccation resistant eggs (*Aedes*, *Ochlerotatus* & *Psorophora*). Pathogens like West Nile virus (WNV) may utilize vector mosquitoes as reservoirs to survive the winter and reemerge the following season. Overwintering adult mosquitoes require sheltered areas that remain above freezing and have high relative humidities, such as caves and urban stormwater tunnels. This overwintering period is the longest period of the mosquito life cycle.

Urban stormwater tunnels in Illinois have been monitored for over a decade including areas in Champaign and Cook counties. In all of these studies, these man-made "caves" tend to have large numbers of *Culex pipiens* Linnaeus and occasionally *Anopheles* species and other Diptera. In subterranean sites, the hibernating mosquitoes tend to aggregate in areas near entrances (man-hole or catchbasin grate), but away from entrance and twilight areas subject to freezing. Mosquitoes may remain in these sites for several months (August to March), relying on fat reserves built up from nectar feeding prior to entering hibernacula. A mile of stormwater tunnel in many areas of Illinois may contain 1,000 - 2,000 *Culex* spp.

*Culex restuans* and *Cx. pipiens* are the primary species in this genus responsible for WNV transmission in Illinois. Although both species overwinter as adults, urban stormwater tunnel habitats are typically occupied by *Cx. pipiens*, whereas hibernacula of *Cx. restuans* are unknown, but may include animal burrows, leaf litter, or hollow trees. Overwintering success appears to be related to the focal reestablishment of transmission of WNV.

In fall, *Cx. pipiens* undergoes hormonally induced diapause partially related to shortening day length (<14.25 hrs in central Illinois). At this time, newly emerged adults stop blood feeding and become nectar feeders; usually engorging on nectar until the abdomen is swollen.

*Cx. pipiens* does not exclusively use stormwater tunnels for hibernacula, but few other sites are as well suited for such large numbers. High humidity and temperatures above freezing but below 50°F provide an optimal range for energy conservation.

West Nile virus spread across the US in less than five years, becoming established in multiple of ecosystems and biomes. This suggests WNV uses a variety of vectors, hosts and over-wintering mechanisms. The prevailing view is that WNV uses mosquitoes both as vectors and as winter maintenance hosts.

Guthrie Cave, Union Co., Illinois. Mosquitoes are typically found in entrance and twilight zones of caves with sufficient moisture - this cave, with a stream emerging from the entrance, represents optimal habitat for overwintering mosquitoes.



Photo by S. Taylor

Locations of six caves sampled for overwintering mosquitoes in southern Illinois.



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## For information

Contact Rich Lampman <richlamp@uiuc.edu> or Steve Taylor <staylor@inhs.uiuc.edu> for more on this study. An 8.5x11 pdf copy of this poster is available at:

<https://neffies.uiuc.edu/staylor/www/rl-ISA08.pdf>

