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MSU FISHERIES & WILDLIFE

SPOTLIGHT

Produced by Graduate Students in the Department of Fisheries & Wildlife at MSU

SPARTANS as Leaders in Global Wildlife Conservation

the future of FW: Getting Kids Outdoors

ALSO INSIDE: Saginaw Bay’s Mysterious Muck, Opening Roads in Nicaragua & More!
Welcome! On behalf of the magazine committee, I am excited to present the sixth issue of FW SPOTLIGHT. This magazine is a product of the Graduate Student Organization (GSO) — it is written, edited and designed by graduate students in the Department of Fisheries and Wildlife at Michigan State University.

This issue of FW SPOTLIGHT demonstrates the diversity of research and outreach efforts being conducted by graduate students in the Department. Features in this issue cover topics ranging from West Nile Virus to youth education and outreach and take place in locales as close as East Lansing and as far as Nicaragua.

Bret Muter is a first-year doctoral student under the direction of Dr. Meredith Gore and Dr. Shawn Riley. He is researching human dimension issues surrounding bovine tuberculosis in Michigan and Minnesota. Contact Bret at muterbre@msu.edu.

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**CONTACT US!**

FW SPOTLIGHT is continually improving and we always appreciate feedback from our readers. Send us an e-mail at fwspotlight@gmail.com to let us know what you think!
A Culex pipiens mosquito collects a blood meal from the photographer’s hand. This insect is one of the culprits involved in the transmission cycle of West Nile Virus. See page 16 for the full story.

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Giraffe at the White Oak Conservation Center in Yulee, Fla.
Photograph by Jessica Kahler

Admiring a red-backed salamander in Paradise, Mich.
Photograph by Dawn Reinhold
The rapid invasion of West Nile virus (WNV) after 1999 westward across the United States has resulted in over 1,000 human deaths and 20,000 non-fatal human cases, as well as countless cases of wild and domestic animal disease. As the virus expands in geographic range, it leaves behind a complex ecological puzzle involving the virus, blood-feeding insects and birds which must interact for human disease to occur.

My dissertation research combined molecular and ecological techniques to incriminate the mosquitoes and birds responsible for maintaining and transmitting WNV. I used trace quantities of blood in the mosquitoes’ abdomen to identify the bird or mammal species the mosquito fed on. I was also able to detect WNV-positive mosquitoes that have human-derived blood meals, thus demonstrating direct evidence of virus exposure to humans.

Diseases shared among wildlife, domestic animals and humans are becoming increasingly common; molecular tools allow researchers to understand these complex disease ecology systems. Knowledge about the transmission cycle in nature allows for the development of management strategies that reduce human health risk to wildlife and mosquito-borne disease. WNV is an example of an emerging disease that recently appeared in the United States and rapidly swept through North and South America (Figure 1).

My dissertation research was part of a larger project studying the epidemiology of WNV transmission in suburban Chicago, Ill., with collaborators from the University of Illinois and the University of Wisconsin-Madison. Part of my research focused on the ecology of WNV transmission and the incrimination of the mosquito and bird species responsible for transmission.

Once infected, not all birds and mosquito species respond the same. Laboratory researchers can infect birds with WNV to observe disease development and determine the ability of a bird species to be a good WNV host. The same is true for mosquito species, which vary in their ability to become infected and then re-infect a subsequent host during feeding.

Additionally, mosquito species vary in their host choice. They tend to take blood meals from some host species more so than others; thus, the ‘preferred’ bird species are exposed to WNV more frequently than ‘avoided’ species.

Although a number of birds have been implicated as important amplification hosts based on laboratory studies, few studies have combined all the important information necessary to critically judge which birds are actually responsible for increasing the virus to high levels in the field.

Learning more about host choice will improve our understanding of which mosquito species are transmitting the pathogen among birds...
and which mosquito species are passing the virus to humans. This information is critical for effective management of WNV, where mosquito control of larval habitat is the most effective technique. Different species of mosquitoes breed in different types of habitat, such as temporary flood water or small containers. Instead of public health agencies attempting to treat all mosquito larval habitat, the results of this research can direct management efforts to target the primary species responsible for WNV transmission.

**MOLECULAR TOOLS TO STUDY WNV TRANSMISSION**

The advent of a molecular tool called the polymerase chain reaction (PCR) in the 1980s generated a suite of applications to the study of wildlife and mosquito and tick-borne diseases. This technique can amplify a region of DNA from a few copies to millions, which allows for the detection of pathogens at very low levels. Researchers can use PCR-based approaches to identify pathogens and species by detecting unique regions of DNA.

One such PCR-based molecular tool is the mosquito blood meal analysis (Figure 2). The goal of the blood meal analysis is to identify the unknown host by analyzing the blood meal in the mosquito’s abdomen. This tool allowed us to investigate the roles of different birds, mammals, and mosquitoes in WNV transmission and offers an improved understanding of how diseases are maintained in nature. We used this molecular tool in our WNV research based in suburban Chicago.

Our research team collected blood-fed mosquitoes from 2005 to 2007 in residential and semi-natural areas using several mosquito traps and collection techniques. The blood-fed mosquitoes were brought to MSU for the molecular analyses, which began with the removal of the blood-fed abdomen and the subsequent isolation of DNA. We then performed PCR to amplify a region of DNA called the cytochrome b gene, after which we used DNA sequencing to determine the unique genetic identity that could lead us to the identification of the species.

**FIGURE 1. WNV TRANSMISSION**

The transmission cycle consists primarily of virus transmission between mosquitoes and birds, where the virus is able to replicate inside an infected bird, which then infects a new mosquito feeding on that bird. Once this WNV cycle amplifies the virus to high levels with many infected individuals, mosquitoes that feed on birds and mammals can become infected by feeding on infectious birds, and then can pass the pathogen to humans and domestic animals. Although mammals, including humans and horses, can become sick and potentially die from WNV, the virus does not replicate inside the blood of mammals to high enough levels to re-infect a mosquito. For these reasons, most mammals are considered “dead-end” hosts because they are not involved in the maintenance of the virus.

**COMBINING MOLECULAR & ECOLOGICAL FACTORS**

Once we identified the species of vertebrate hosts that mosquitoes fed upon, we were able to learn more about transmission ecology. However, as feeding patterns are influenced by host availability, we first needed to measure bird abundances in order to identify which bird species are preferred, avoided or used in proportion to availability. Therefore, while collecting mosquitoes, we also performed bird surveys to estimate relative densities.

We used several calculations to determine mosquito preference for a bird host and the contribution of a particular species of bird to the transmission of WNV. Using these molecular and ecological approaches, we identified the top three bird species responsible for WNV transmission and amplification as the
American robin, blue jay and house finch. Combined, these three species were responsible for 66% of the amplification of WNV in our study region. We are also able to identify the roles of different mosquito species in the transmission system. Of the 661 *Culex pipens* mosquitoes from which we identified blood meals, 83% were avian; this confirms their role as the primary host amplifying the virus to high levels. However, we noted significantly more mammal-feeding by *Cx. pipiens* than is expected of this species, including one individual that tested WNV-positive and contained a human-derived blood meal. This finding is the first direct evidence that *Cx. pipiens* is capable of being the amplification mosquito species and the species responsible for human exposure, probably acquiring WNV from an infectious bird and subsequently transmitting WNV to a human during the next blood meal.

We provided our collaborators at the Connecticut Agricultural Experiment Station with the DNA from our *Cx. pipiens* specimens. They investigated a potential genetic basis for the patterns of avian and mammal feeding that we observed. Through a molecular approach, our collaborators determined that individual *Cx. pipiens* with mammal-derived blood meals were more likely to share ancestry with a type of mosquito that is known to have higher inclination for feeding on mammals. This is one of the first reports of this type of *Cx. pipiens* in the Midwest and increases our understanding about mechanisms of WNV spillover into humans.

**CONCLUSION**

Molecular tools are an important asset to ecologists studying disease at the wildlife, domestic animal and human interface. This multidisciplinary approach requires collaboration among experts in different fields and is necessary in the attempt to fully understand complex disease ecology systems. For example, my dissertation research combined the traditional fields of wildlife ecology, medical entomology and microbiology to address questions related to the transmission of a pathogen among birds, mosquitoes and humans. My findings incriminate a particular species of mosquito that is possibly responsible for both increasing WNV to high levels as well as passing the virus to humans.

With this knowledge, public health agencies can allocate effort appropriately and target specific breeding habitat that will prevent *Cx. pipiens* from reaching high abundances. The primary breeding locations for this container-breeding mosquito are catch basins, which are designed to receive and send rain water to the nearest stream through underground pipes. Other types of urban breeding habitats for *Cx. pipiens* mosquitoes include bird baths, flower pots, buckets and any container capable of holding a small amount of water. You can do your part in controlling mosquito-borne diseases in your own backyard by removing these containers or ensuring they are treated with mosquito larvicide.

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**FIGURE 2. MOSQUITO BLOOD MEAL ANALYSIS**

The goal of the blood meal analysis is to identify the unknown host by analyzing the blood meal in the mosquito’s abdomen. This tool allowed us to investigate the roles of different birds, mammals and mosquitoes in WNV transmission and offers an improved understanding of how diseases are maintained in nature.

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