Report for 2004ND46B: Effects of West Nile Virus Infection, Immune Function, and Age on Female Yellow-headed Blackbird (Xanthocephalus xanthocephalus) Reproduction.

- Conference Proceedings:
  - Newbrey, J. (2004) “WEST NILE VIRUS ANITBODIES IN CENTRAL NORTH DAKOTA ICTERIDS.” Presented at the Cooper Ornithological Society’s 2004 Annual Meeting, May 6-8, La Crosse, Wisconsin.

Report Follows

EFFECTS OF WEST NILE VIRUS INFECTION, IMMUNE FUNCTION, AND AGE ON FEMALE YELLOW-HEADED BLACKBIRD (*XANTHOCEPHALUS XANTHOCEPHALUS*) REPRODUCTION
(Partial Renewal)

DESCRIPTION OF THE CRITICAL WATER PROBLEM

Recent high water levels and canalization of water resources (i.e., Garrison Diversion) in North Dakota have resulted in an increase of aquatic habitats for many wildlife species. Current water conditions correspond with increased numbers of wetland breeding birds and increased habitat for breeding mosquitoes. Because birds often serve as intermediate hosts for mosquito borne diseases, increased populations of birds and mosquitoes could impact the ecology, rate of emergence, and persistence of diseases in humans and wildlife. The recent spread of WNV into the state has produced a need for research to study the influence of the virus on wetland wildlife in North Dakota.

The North Dakota Department of Health reported the first cases of WNV in the state in the summer of 2002. The first bird to test positive for WNV was an American crow (*Corvus brachyrhynchos*) found on July 14th, and the first positive human cases were reported on August 28th. There were 19 human cases of WNV and 2 deaths in the state in 2002. In 2003, the virus was much more widespread, with 422 human cases and 4 deaths. This year alone, 788 bird carcasses have been tested in the state and 189 were WNV positive.

Because stagnant water in wetlands is ideal breeding habitat for mosquitoes, wildlife associated with these habitats may suffer high rates of WNV infection. The recent arrival of WNV into the state necessitates a study of the prevalence and immunological impact of WNV on native North Dakota wetland species. Most research on the virus has focused on using carcasses of birds as a surveillance system for detecting the spread of WNV across North America. No published research has been conducted on a living population of free-ranging birds. Failure of biologists to adequately address disease emergence in free-ranging wildlife may lead to diminished geographic distributions and populations declines (Friend et al. 2001).

The Missouri Coteau of central North Dakota has many small prairie wetlands, which provide essential foraging and breeding habitat for many species of birds. Yellow-headed blackbirds are an ideal species to study WNV infection because they breed in high-density wetland colonies throughout the Coteau. Establishing rates of WNV infection in yellow-headed blackbirds is necessary to determine the vulnerability of this wetland dwelling species and the influence of WNV infection on reproduction. Information gathered on WNV for this study can also be used to model and predict potential impacts of the virus on other species of wetland birds.

KEY LITERATURE

West Nile virus is a mosquito-borne virus that was first diagnosed in North America in 1999 (Rappole et al. 2000). Since that time, the virus has spread across the United States and into Canada. Because the virus can cause fatal meningitis, it has become a national health concern for human populations, an economic concern for domestic animal losses, and a concern for the status of free-living bird populations (Campbell et al. 2002). Birds are one of the principal hosts of WNV (Rappole et al. 2000), with more than 138 species of wild birds diagnosed with the virus in the United States since 1999 (CDC unpub. data). Most of the information we have on the prevalence and distribution of the virus is based on information from symptomatic birds (i.e., sick or dead), which can bias estimates of potential outbreaks if asymptomatic birds have survived infection or act as carriers of the virus. By testing for the presence of antibodies to WNV in yellow-headed blackbirds and monitoring reproductive performance, I will be able to assess the degree to which a free-living population has been exposed to the virus, the non-lethal effects of WNV infection on reproduction, and the potential for blackbirds to serve as carriers of WNV.

WNV could influence yellow-headed blackbird reproduction by compromising female immunity. When a bird suffers immune system stress, resources are allocated away from nonessential processes, such as growth and reproduction, and are reallocated to activities directly related to survival (Lochmiller and Deerenberg 2000). For example, depressed female immunity is known to decrease the diversity and concentration of carotenoids in egg yolks (Saino et al. 2002). Carotenoids are biologically active, lipid-soluble pigments synthesized by plants and photosynthetic microorganisms, which animals must obtain from their diet (Blount et al. 2000). In developing avian embryos, carotenoids in the yolk protect vulnerable tissues against damage caused by free radicals, by-products of normal metabolism and immune defense, which can cause extensive DNA, protein, and lipid damage (Surai et al. 2001). Because carotenoids are derived solely from the female, they reflect the quality of the maternal diet prior to egg laying.

As powerful antioxidants and immunostimulants, carotenoids are also incorporated into the sexual signals of many animals and are thought to indicate individual health (Blount et al. 2000). Yellow-headed blackbirds depend on carotenoids for their brightly colored yellow plumage. Second year (SY) female yellow-headed blackbirds can be distinguished from older, after second year (ASY) females because they have smaller patches of yellow feathers and are paler in their head, neck and breast regions (Crawford and Hohman 1978). These differences in plumage may reflect a female's ability to obtain carotenoids from surrounding habitats and therefore reflect her ability to deposit carotenoids into her eggs (Royle et al. 2001). Female age could also have substantial impacts on resource allocation and immune function. Young females may allocate more physiological resources to growth than mature females, and therefore allocate less to reproduction and immunity.

Maternal tradeoffs exist between the use of carotenoids for physiological functions, the expression of sexual signals, and investment into eggs (Saino et al. 2002). Yellow-headed blackbirds are an ideal species to study maternal tradeoffs between reproduction and self-maintenance, because females can easily be separated into 2 age classes and they allocate carotenoids both to plumage and eggs. Also, the high density of conspicuous, easily accessible nests in small North Dakota wetlands makes yellow-headed blackbirds an ideal species for study of WNV infection, immune response, and carotenoid concentrations in breeding females and their offspring.

SCOPE AND OBJECTIVES

The overall objective of this project is to determine the effects of female age and infection with West Nile virus on yellow-headed blackbird (*Xanthocephalus xanthocephalus*) maternal investment into eggs. The specific objectives of this project are to identify the prevalence of WNV in a free-living population of yellow-headed blackbirds, to quantify variation in immune function of female blackbirds, and to measure the relationship between female immune function and age on carotenoid allocation to eggs. These objectives will allow us to evaluate potential relations between wetland bird WNV infection and increased aquatic habitat for breeding mosquitoes in North Dakota.

METHODS, PROCEDURES, AND FACILITIES

Female yellow-headed blackbirds will be captured using mist-nets in order to collect blood samples to assess WNV antibody production and to measure variation in immune function using non-lethal immune challenges. Blood serum will be tested for WNV antibodies using competitive enzyme-linked immunoabsorbent assay (ELISA). We will follow protocol specifically designed to detect WNV antibodies in blood serum from avian species (Dr. Barry Beaty, Colorado State University, pers. comm.). We will quantify variation in female immune function at both the cell-mediated (i.e., white blood cell) and humoral (i.e., antibody production) levels using the methods described in Casto et al (2001). To assess cell-mediated immunity, we will measure differential-cutaneous swelling between wings of 30 females injected with a harmless plant protein (phytohemaglutinin - PHA) in the right wing and saline solution in the left. We will assess variation in humoral immune response by quantifying the antibody production of 30 females injected with sheep red blood cells (a novel, non-lethal antigen). Prior to release, each female will be categorized as either SY or ASY and will be banded with a standard Fish and Wildlife Service aluminum band along with a unique color-band combination for individual field identification.

We will locate and monitor nests of marked females to assess maternally allocated carotenoids in eggs and offspring survival and growth. The third-laid egg will be removed from each nest for carotenoid analysis, which insures the detection of differences among females and not variation in carotenoid allocation due to egg laying order. Yolks will be separated from the egg and the carotenoids will be extracted from the yolk using the methods described in Surai and Speake (1998). We will analyze carotenoids with High Performance Liquid Chromatography (HPLC) using a reverse-phase column. We will assess the concentration of carotenoids by comparing my samples to a calibration curve obtained from known concentrations of carotenoids. After sampling the third egg, we will monitor each nest at 3-day intervals to determine hatching success and nestling growth rates (i.e., body mass, tarsus length, and wing length).

This study is being conducted on several wetlands located within a five square mile area of the Missouri Coteau region of central North Dakota (Stutsman County). Central North Dakota has one of the highest concentrations of yellow-headed blackbirds in North America (Twedt and Crawford 1995). In addition, Stutsman County has one of the highest numbers of positive avian West Nile virus cases in central North Dakota (CDC unpub. data).

My advisor, Dr. Wendy Reed will provide field facilities in Stutsman County for all field data collection (i.e., equipment storage, animal care, and lodging for myself and my assistant). Lab analysis of WNV antibodies and carotenoid concentrations will be conducted at NSDU.

ANTICIPATED RESULTS AND DELIVERABLES

This study will provide essential information on the prevalence and immunological impact of WNV on a North American avian species. Infection with the virus can be lethal, however, the degree to which birds are adversely affected varies across species and even between individuals within a species (Rappole et al. 2000). By testing for the presence of antibodies to WNV in yellow-headed blackbirds, we will be able to assess the vulnerability and degree of virus exposure in a free-living population of wetland dwelling birds. We will also be able to evaluate potential influences of current high-water conditions on breeding populations of mosquitoes and avian WNV infection rates.

Many wildlife pathogens cause non-lethal physiological and reproductive effects that remain poorly understood. This study will quantify the immunological costs and maternal tradeoffs associated with exposure to a non-lethal antigen. Because female birds allocate essential resources to eggs, exposure to pathogens can shift maternal resources away from reproduction. This seemingly small, non-lethal effect influences the survival of offspring and can therefore cause population level effects in the next generation.

The research conducted for this study will comprise a Ph.D. dissertation. Results will be presented at an American Ornithologist Union conference and will ultimately be submitted for publication to a prestigious peer-reviewed journal.

PROJECT PROGRESS

During my first field season from May to August of 2003, I collected 51 eggs and 20 feather samples to use for yellow-headed blackbird carotenoid quantification. I conducted immune challenges on five females, but the majority of the immunity and nest performance data will be collected in upcoming field seasons. I also collected blood samples for WNV antibody detection from 44 yellow-headed blackbirds, 18 grackles, two red-winged blackbirds, and one western meadowlark. This fall I have tested the 65 blood samples for WNV antibodies and I detected antibodies in only two individuals, one red-winged blackbird and one western meadowlark.

I have two hypotheses to explain why I did not find WNV antibodies in yellow-headed blackbirds or common grackles. The first is that WNV could be lethal in free-living individuals of these two species. In a recent study by Komar et al. (2003), experimentally infected common grackles were shown to have a 33% death rate within four days of being infected with WNV. Perhaps death rates are closer to 100% in less hospitable natural conditions and that is why I was unable to detect individuals with antibodies. In contrast to common grackles, all of the red-winged blackbirds that were experimentally infected with WNV survived infection, indicating that the species is able to survive WNV infection by producing antibodies (Komar et al. 2003).

High death rates in yellow-headed blackbirds and common grackles due to WNV infection could influence human health. Bird species that suffer high death rates associated with WNV have been found to have high viremia levels circulating in their blood streams (Komar et. al. 2003). They also have the highest probability of passing the virus to a mosquito vector and therefore to a human. If the virus is lethal in yellow-headed blackbirds and common grackles, infected individuals may act as virus reservoirs in central North Dakota.

The loss of large numbers of yellow-headed blackbirds and common grackles due to lethal WNV infection would have ecological ramifications in the food-web dynamics of the prairie wetlands of North Dakota. Eggs, young, and adult birds of both species are an invaluable food source to predatory birds and mammals. In addition, WNV induced yellow-headed blackbird population crashes could result in a population explosion of red-winged blackbirds. As the most abundant songbird in North America, red-winged blackbirds cause extensive crop damage in North Dakota annually. Yellow-heads help to control red-winged blackbird numbers by excluding red-wing breeding pairs from their colonies and competing for natural food sources.

My second hypothesis is that WNV infection rates are low in yellow-headed blackbirds and common grackles. If infection rates are low, perhaps I did not have a large enough sample size to detect WNV infected individuals. This hypothesis seems unlikely since one out of two red-winged blackbirds and the only western meadowlark sampled had WNV antibodies. Since all sampled birds were collected within a five square mile area, it would seem infection rates were actually high in my study area.

I am very intrigued by my preliminary findings and will continue to collect more information on WNV infection in yellow-headed blackbirds and common grackles in my upcoming field seasons. I also hope to collaborate with another university to experimentally infect yellow-headed blackbirds with WNV in captivity to better understand how the species responds to infection.

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