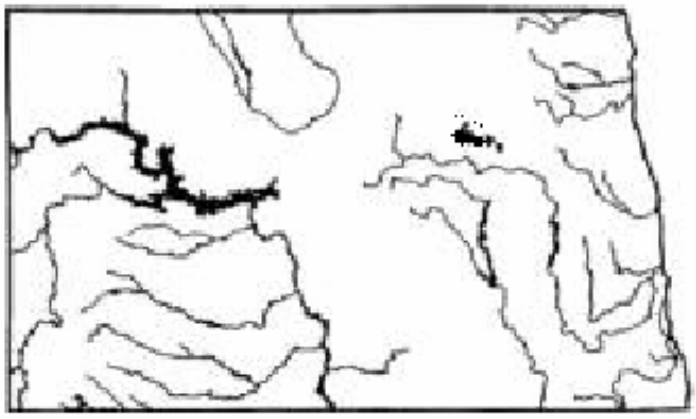

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Abstract

West Nile virus (WNV) has resulted in the deaths of tens of thousands of North American birds, but population-level effects of the virus remain poorly understood. Breeding birds and nestlings may be particularly vulnerable to WNV because many species nest in close proximity to mosquito breeding habitats and newly hatched chicks lack defensive mechanisms against mosquitoes. To determine the prevalence and potential reproductive impacts of WNV in central North Dakota blackbird populations, we captured and tested 171 free-living icterids for WNV-specific antibodies during the 2003 and 2004 breeding seasons. During the 2004 breeding season, we also trapped and tested 291 female *Culex tarsalis*, a known WNV vector, for WNV RNA. Five icterids were positive for WNV antibodies, including two adult Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*), an adult Common Grackle (*Quiscalus quiscula*), an adult Red-winged Blackbird (*Agelaius phoeniceus*), and a juvenile Western Meadowlark (*Sturnella neglecta*). In contrast, all *C. tarsalis* were negative for WNV RNA. Our results suggest that WNV infection rates were low in our study area during the 2003 and 2004 breeding seasons, with 3.7 and 2.6% of captured icterids expressing WNV-specific antibodies respectively. However, difficulty in capturing infected individuals and virus-induced lethality could influence WNV infection rate estimates in free-living bird populations.

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Introduction

Exotic infectious diseases can have devastating effects on the distribution and abundance of naïve wildlife species (Friend et al. 2001). West Nile virus (WNV) is an exotic disease that was introduced into North America in 1999 and has resulted in the deaths of tens of thousands of birds (Marra et al. 2004). The natural cycle of WNV involves *Culex* species mosquitoes as the principle vectors and birds as the principle hosts, although humans, horses, and other mammals can become incidental hosts (Lanciotti et al. 2000). Because the virus can be fatal, outbreaks of the virus have become a national health concern for the human population, an economic concern for domestic animal losses, and a concern for the status of free-living wildlife populations (Campbell et al. 2002). For birds, WNV infection can be lethal, but the degree to which birds are adversely affected varies among species and even between individuals within a species (Komar et al. 2003).

Lethal avian outbreaks of WNV in North America have raised concerns for the status of free-living bird populations (Marra et al. 2004). Studies of the impacts of WNV on North American bird populations have mainly focused on the American Crow (*Corvus brachyrhychos*), because the species exhibits high rates of WNV-induced lethality (Peterson et al. 2004). For

example, Yaremych et al. (2004) found a 68% WNV-attributed death rate in a radio-marked population of crows in Illinois in 2002. In 2003, Caffrey et al. (2005) found similar WNV death rates (65%) in a color marked population of American Crows in Oklahoma. West Nile virus has caused deaths in at least 198 species of birds in North America (Komar 2003), but population-level impacts of the virus continue to be poorly understood.

North American wetland breeding birds and nestlings may be particularly vulnerable to WNV because they nest in close proximity to mosquito breeding habitats and newly hatched chicks lack defensive mechanisms against mosquitoes. Central North Dakota wetlands are ideal locations in which to study the prevalence and potential reproductive impacts of WNV on free-living bird populations. Small prairie wetlands throughout the region provide preferred breeding habitats for many species of birds and mosquitoes, including icterids and *Culex* mosquitoes. Several abundant icterid species, including Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*), Red-winged Blackbirds (*Agelaius phoeniceus*), and Common Grackles (*Quiscalus quiscula*), nest in or adjacent to prairie wetlands. Also female *Culex tarsalis* mosquitoes, known vectors of WNV in North Dakota (Bell et al. 2005), lay their eggs in highly organic wetland water (Crans 2004). *Culex tarsalis* is one of the most efficient laboratory vectors of WNV in North America, even when compared to four other members of the genus *Culex* (Goddard et al. 2002).

Objective

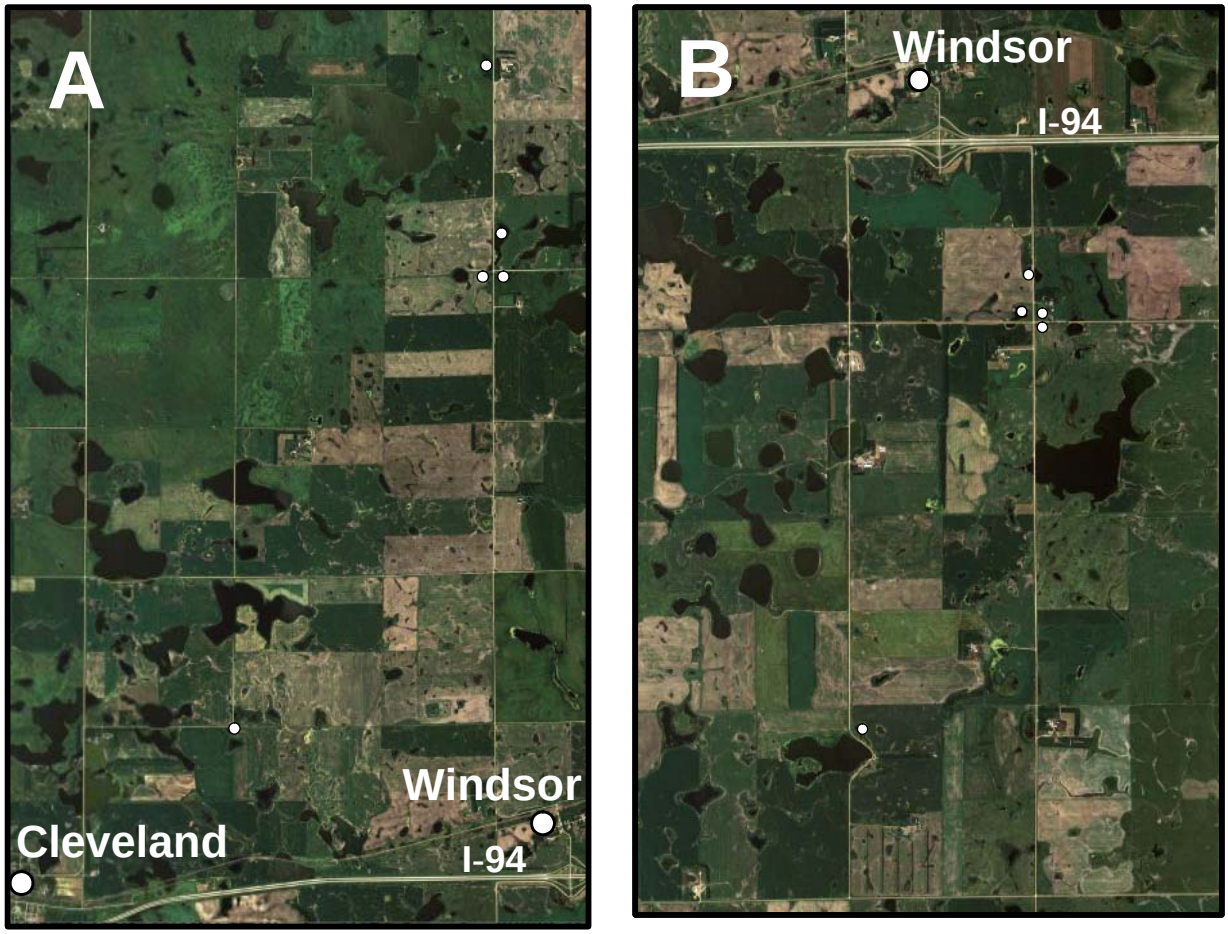
In light of concerns regarding the status of North American bird populations, we captured adult, hatch year, and nestling central North Dakota icterids and tested them for WNV-specific antibodies. Specifically, our objective was to determine if antibody positive blackbirds were

present in central North Dakota during the early summer breeding season. Sampling during the icterid breeding season also allowed us to test the hypothesis that nestling blackbirds are particularly vulnerable to the virus because they are confined to the nest, lack protective feathers, and have naïve immune systems. We also trapped mosquitoes to determine if *Culex tarsalis* was present in our study area.

Methods

This study was conducted on 10 wetlands in Stutsman County, North Dakota, during the icterid breeding season, from 15 May to 16 July in 2003 and 2004 (Fig. 1). A total of 170 free-ranging icterids (132 adults, 5 hatch year, and 33 nestlings) were captured and tested for WNV antibodies. Food-baited-live traps were used to capture 56 blackbirds, including Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus,* n = 13), Common Grackles (*Quiscalus quiscula,* n = 38), Red-winged Blackbirds (*Agelaius phoeniceus*, n = 3), and Brown-headed Cowbirds (*Molothrus ater*, n = 2). A nest trap was used to capture 81 additional female Yellow-headed Blackbirds. Thirty three nestling Yellow-headed Blackbirds between 8 and 11 days old were sampled from 27 nests. Nineteen of the nestlings were offspring of females that were also tested for WNV antibodies. Additional samples were obtained from a road-kill hatch-year Western Meadowlark (*Sturnella neglecta*) and three House Sparrows (*Passer domesticus*, an adult and two juveniles), but they were not included in estimates of WNV antibody-positive icterids.

A blood sample (< 150 µl) was collected via venous puncture at the brachial vein from all captured individuals. All Yellow-headed Blackbirds were banded as part of a concurrent research project and the outermost tail feather of all other birds were clipped to prevent re-sampling. Whole blood was kept in heparinized microcapillary tubes on ice for no longer than 6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hours before it was centrifuged for 5 minutes at 6000 x g. The serum portion was removed and stored at –20ºC until lab analysis.

To determine if *C. tarsalis* mosquitoes were present in the study area, mosquitoes were sampled using carbon dioxide-baited traps (American Biophysics Corp., North Kingstown, RI) adjacent to two study wetlands from 9 June to 15 July 2004. Traps were run for three consecutive 24-hour periods before mosquitoes were collected and promptly frozen at –20ºC (Bell et al. 2005).

Avian serum samples were tested for WNV antibodies using competitive enzyme-linked immunoabsorbent assay (ELISA) using protocols specifically designed for wild birds (Blitvitch et al. 2003). The ELISA, using WNV-specific monoclonal antibodies, provides a non-lethal, rapid, and inexpensive technique for monitoring WNV infection in wild bird populations (Blitvitch et al. 2003). Serum samples from an infected horse and from normal chickens were used as positive and negative controls (Blitvitch et al. 2003). A percent inhibition value of >30% was considered to represent a serum sample positive for WNV antibodies (Blitvitch et al. 2003).

Approximately 25,000 mosquitoes were collected during the summer of 2004 (Table 1). A total of 291 female *C. tarsalis* individuals were recovered and tested for WNV RNA at the University of North Dakota using Reverse Transcriptase Polymerase Chain Reaction (Lanciotti et al. 2000).

Results

Of the 170 icterids sampled in 2003 and 2004, four individuals were positive for WNV antibodies (Fig. 2, Table 2). In 2003, one male Red-winged Blackbird tested positive for the antibodies out of 53 blackbirds sampled (1.9%). In 2004, three blackbirds tested positive out of

Table 1. Eight species of mosquitoes collected in central North Dakota during the 2004 icterid breeding season. A subset of 420 individuals were identified to species out of approximately 25,000 individuals collected.

Species	Date of first collection	# individuals	% sample
Aedes vexans	6/9/2004	327	77.9
Anopheles walkeri	6/9/2004	41	9.8
Ochlerotatus dorsalis	6/9/2004	23	5.5
Anopheles quadrimaculatus	6/9/2004	9	2.1
Culex tarsalis	7/7/2004	6	1.4
Ochlerotatus flavesens	7/13/2004	9	2.1
Culiseta inornata	7/13/2004	4	1.0
Culiseta morsitans	7/13/2004	2	0.5

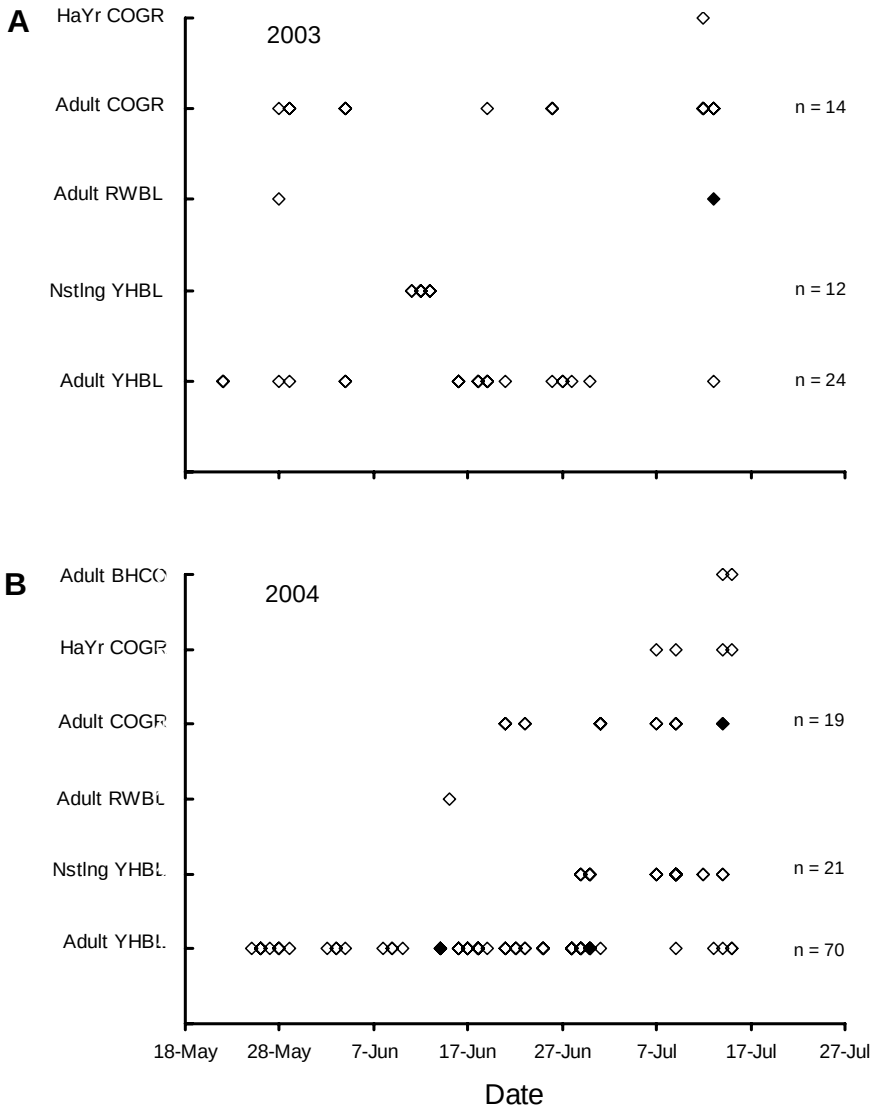

Figure 1. Trapping dates and the occurrence of birds with West Nile virus antibodies (closed symbols) during the A. 2003 and B. 2004 icterid breeding seasons. Sample sizes are given for species with overlapping data points. Abbreviations are as follows; HaYr = hatch year, Nstlng = nestling, COGR = Common Grackle, RWBL = Red-winged Blackbird, YHBL = Yellow-headed Blackbird, and BHCO = Brown-headed Cowbird.

Table 2. Icterid species tested for West Nile virus (WNV) antibodies in central North Dakota during the 2003 and 2004 icterid breeding seasons. The number tested and the number of WNV-antibody positive individuals are reported for each species.

	2003		2004	
Species	Tested	WNV positive (%)	Tested	WNV positive (%)
Yellow-headed Blackbird	36	0 (0.0)	91	2 (2.2)
Common Grackle	15	0 (0.0)	23	1 (4.3)
Red-winged Blackbird	2	1 (50.0)	1	0 (0.0)
Brown-headed Cowbird	-	-	2	0 (0.0)
Total	53	2 (1.9)	117	3 (2.6)

117 sampled (2.6%), two adult female Yellow-headed Blackbirds and one adult Common Grackle. The juvenile Western Meadowlark collected in 2003 and two House Sparrows (an adult and a juvenile) collected in 2004 also tested positive for WNV antibodies. All of the *C. tarsalis* mosquitoes sampled during the 2004 icterid breeding season tested negative for WNV RNA.

Discussion

The occurrence of WNV antibodies was low in the study area during the icterid breeding seasons in 2003 and 2004, with 1.9 and 2.6% of blackbirds having antibodies respectively. These rates of antibody positive birds were slightly lower than those found in other living bird populations that have recently been monitored for WNV. In 2002, Ringia et al. (2004) studied the prevalence of WNV antibodies in 81 species of North American birds in Illinois and found an overall rate of 5.3% (94 out of 1,784 tested). Of the 39 Red-winged Blackbirds they sampled, three individuals (7.7%) were antibody positive (Ringia et al. 2004). Similarly, the North Dakota Department of Health tested 617 live birds for WNV antibodies during the summer of 2004, and they found 36 positive individuals (5.8%, North Dakota Department of Health unpubl. data).

In the present study, several birds had WNV antibodies despite the lack of WNV RNA positive female *C. tarsalis*, potentially due to off site infection or the timing of antibody sampling. Four of the antibody-positive adult blackbirds may have been infected with WNV while they where on their wintering grounds. Naturally infected Rock Pigeons (*Columba livia*) have detectable antibody levels up to 15 months after initial infection (Gibbs et al. 2005), so migratory birds exposed to WNV in the southern United States may have detectable antibody levels after reaching their breeding grounds. However in my study, antibody positive birds were

not detected until half-way through the breeding season (14 June), and one adult non-migratory House Sparrow and two juvenile birds (a Western Meadowlark and a House Sparrow) were also WNV-antibody positive, suggesting local infection. Both birds and mosquitoes were sampled just prior to the peak of *C. tarsalis* mosquitoes, so that I could determine if antibody positive blackbirds were present in central North Dakota during the early summer icterid breeding season. Therefore, early sampling may have contributed to the low number of birds and mosquitoes detected with WNV.

Changes in bird behavior associated with WNV infection and lethal infection could also reduce the number of WNV antibody positive birds detected in free-living bird populations. Most studies of WNV in living bird populations depend on mist nets or live traps to capture individuals to test for antibodies. However, infected individuals may exhibit non-lethal disease symptoms that could preclude them from capture (i.e., lethargy, hiding, etc.). For some species WNV may be lethal and antibody levels may not be detectable before individuals succumb to the virus. For example, captive Common Grackles have a 33% death rate within four days of being experimentally infected with WNV (Komar et al. 2003), suggesting that some Common Grackles are unable to survive WNV infection long enough to produce antibodies.

Little is known about the influence of WNV on the reproductive success of North American bird populations. In this study, I sampled 33, 8 to 10 day-old nestling Yellow-headed Blackbirds, which were confined to the nest, partially naked, and therefore potentially at high risk of WNV exposure, but all were WNV-antibody negative. However, both of the WNV antibody-positive female Yellow-headed Blackbirds experienced nest failure in 2004. Although this is anecdotal evidence, additional studies including long-term monitoring of marked populations are needed to help elucidate both the lethal and non-lethal impacts of WNV on North

American bird populations.

Conclusion

Our results suggest that WNV infection rates were low in our study area in Stutsman County, North Dakota during the 2003 and 2004 breeding seasons, with 3.7 and 2.6% of captured icterids expressing WNV-specific antibodies respectively. However, our infection rates may underestimate those infected due to difficulty in capturing infected individuals and virus-induced lethality. Further research, including long-term monitoring of marked populations, is needed to help elucidate both the lethal and non-lethal impacts of WNV on North Dakota bird populations

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