West Nile virus and sage-grouse: What more have we learned?

David E. Naugle, Cameron L. Aldridge, Brett L. Walker, Kevin E. Doherty, Marc R. Matchett, Jock McIntosh, Todd E. Cornish, and Mark S. Boyce

Abstract West Nile virus (WNv) has emerged as a new issue in the conservation of native avifauna in North America. Mortality associated with WNv infection decreased survival of female greater sage-grouse (*Centrocercus urophasianus*) by 25% across 4 populations in Wyoming and Montana, USA, and Alberta, Canada, in 2003. In 2004 WNv spread to populations in Colorado and California, and female survival in late summer was 10% lower at 4 sites with confirmed WNv mortalities (86% survival) than at 8 sites without (96%). We still have no evidence that sage-grouse show resistance to the virus. The 2004 WNv season was not the catastrophe that many had predicted, and the decrease in prevalence of infection and mortality in sage-grouse, humans, and horses (except in California) has left many wondering if the worst has past. Evidence suggests that risk of infection was low in 2004 because unseasonably cool summer temperatures delayed or reduced mosquito production. Moreover, mortalities occurred 2–3 weeks later in 2004 than in 2003, and the shift to later timing was consistent between years at sites where WNv reduced survival both years. Mosquito surveillance data indicated a sharp decline in prevalence and infection rate of adult *C. tarsalis* in southeast Alberta, the most northern latitude where WNv reduced survival, in 2003 but not in 2004. A full understanding of the implications of WNv for sage-grouse requires a long-term, coordinated monitoring strategy among researchers and a sensitivity analysis to evaluate the role of WNv in population viability. Epidemiological research examining the prevalence and ecology of the virus among reservoir hosts is crucial.

Key words *Centrocercus urophasianus*, emerging infectious disease, monitoring, population decline, sage-grouse, survival, West Nile virus

Emerging infectious diseases pose a serious threat to wildlife conservation (Daszak et al. 2000), yet often little is known about consequences of emerging infectious diseases for populations of sensitive or declining native species. Evaluating impacts of emerging infectious diseases to wildlife populations is difficult because monitoring strategies rarely quantify rates of mortality. Since its emergence in New York in 1999, West Nile virus (WNv) rapidly spread west across North America, reaching the west coast in 2004 (Estrada-Franco et al. 2003, Centers for Disease Control and Prevention 2004). Even so, other than data for the American crow (*Corvus brachyrhynchos*) (Caffrey et al. 2003, 2005), population-level consequences of WNv for native North American birds remain virtually unknown (Marra et al. 2004).

Since 2003 WNv has emerged as a conservation

Address for David E. Naugle, Kevin E. Doherty, and Brett L. Walker: Wildlife Biology Program, College of Forestry and Conservation, University of Montana, Missoula, MT 59812, USA; e-mail for Naugle: dnaugle@forestry.umt.edu. Address for Marc R. Matchett: U.S. Fish and Wildlife Service, Charles M. Russell National Wildlife Refuge, Lewistown, MT 59457, USA. Address for Jock McIntosh: Alberta Environment, Edmonton, AB T5K 2J6, Canada. Address for Todd E. Cornish: Wyoming State Veterinary Laboratory, Department of Veterinary Sciences, University of Wyoming, Laramie, WY 82070, USA. Address for Mark S. Boyce and Cameron L. Aldridge: Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.

concern for greater sage-grouse (*Centrocercus urophasianus*) and Gunnison sage-grouse (*Centrocercus minimus*). Previously widespread, sage-grouse have been extirpated from 44% of their original range in western North America (Schroeder et al. 2004), with an estimated range-wide population decline of 45–80% and local declines of 17–92% (Connelly and Braun 1997, Braun 1998, Connelly et al. 2000*a*, Aldridge and Brigham 2003). Habitat loss and degradation already threaten sage-grouse populations (Braun 1998; Connelly et al. 2000*a*,*b*; Aldridge and Brigham 2002; Knick et al. 2003). West Nile virus was first confirmed in greater sage-grouse in July 2003 in the northern Powder River Basin in northeast Wyoming (Naugle et al. 2004). In that year WNv mortality contributed to a 25% decline in survival of 4 populations of radiomarked birds in Alberta, Wyoming, and Montana (Naugle et al. 2004). Late-summer survival of greater sage-grouse in the northern Powder River Basin was markedly lower at 1 site with confirmed WNv mortalities (20% survival) than at 2 sites without (76% survival) (Walker et al. 2004). Moreover, declines in male and female lek attendance at the WNv site in spring 2004 indicated that outbreaks have threatened local populations with extirpation (Walker et al. 2004). Arthropod surveillance in the northern Powder River Basin in 2003 indicated that the most likely mode of transmission was the mosquito (*Culex tarsalis*), a highly competent vector of WNv (Reisen and Reeves 1990, Goddard et al. 2002, Naugle et al. 2004).

In 2004 researchers used published protocols (Walker et al. 2004) to monitor 12 populations of radiomarked sage-grouse for WNv-related mortality. This collaboration is the first range-wide attempt to document the impact of WNv on survival in a declining North American avian species. Our purpose is to enhance future monitoring efforts by synthesizing our current understanding of WNv in sage-grouse.

Methods

Study sites

We surveyed researchers conducting sage-grouse studies to determine whether they had monitored their radiomarked birds for WNv in 2004. Investigators from 12 sites provided survival data from birds monitored 1 July–30 September 2004, except at 4 sites where monitoring ended earlier (Figure 1; Site 2, 17 September; Site 3, 12 September; Site 6, 10 September; Site 11, 24 September). Yearling and adult females were captured using rocket nets, spotlighting (Wakkinen et al. 1992), or walk-in traps and fitted with necklace-type radiotransmitters with mortality switches. Monitoring birds at 2–3-day intervals increases the probability that dead birds are found before being scavenged, which in turn increases the number of carcasses that can be tested for WNv (Walker et al. 2004). Because intensity of monitoring was dictated in part by the study objectives at individual sites, birds from 12 sites in this study were monitored at intervals ranging from 2–8 days ($\bar{x}$ = 4.0 days, SE = 0.7). We used interval length, number of mortalities tested for WNv, and number of mortalities that tested positive to assess whether monitoring intensity was sufficient to warrant inclusion of survival estimates from individual sites into analyses. We classified sites into 3 categories: 1) sites with dead birds that tested positive for WNv, 2) sites that tested dead birds with no indication of WNv, and 3) sites with no mortalities during the monitoring timeframe. We excluded data from 3 sites at which mortalities during late summer were not tested for WNv.

Testing dead birds for WNv

Dead birds underwent complete necropsies and microscopic examination of routine tissues by histopathology at veterinary laboratories. Each carcass was tested for WNv using 2 tests, Real Time Polymerase Chain Reaction (Shi 2001) and immunohistochemistry (Kiupel et al. 2003). Select cases positive for WNv were confirmed by isolation of the virus from 1 or more tissues (brain, heart, kidney, or bone marrow) in Vero cell cultures (Steele et al. 2000).

Estimating survival

We calculated survival for each site as the number of marked individuals alive at the end of the monitoring period divided by number of birds monitored. We used only birds followed throughout the entire period in survival estimates. We used Mann-Whitney *U* tests to compare survival among groups of study sites. We compared survival at sites with dead birds that tested positive for WNv to those sites where dead birds were tested but with no indication of WNv. We also compared survival at infected and uninfected sites by comparing sites with dead birds that tested positive for WNv to those where dead birds were tested but with no indication of WNv and at sites where no mortalities were observed during the monitoring timeframe.

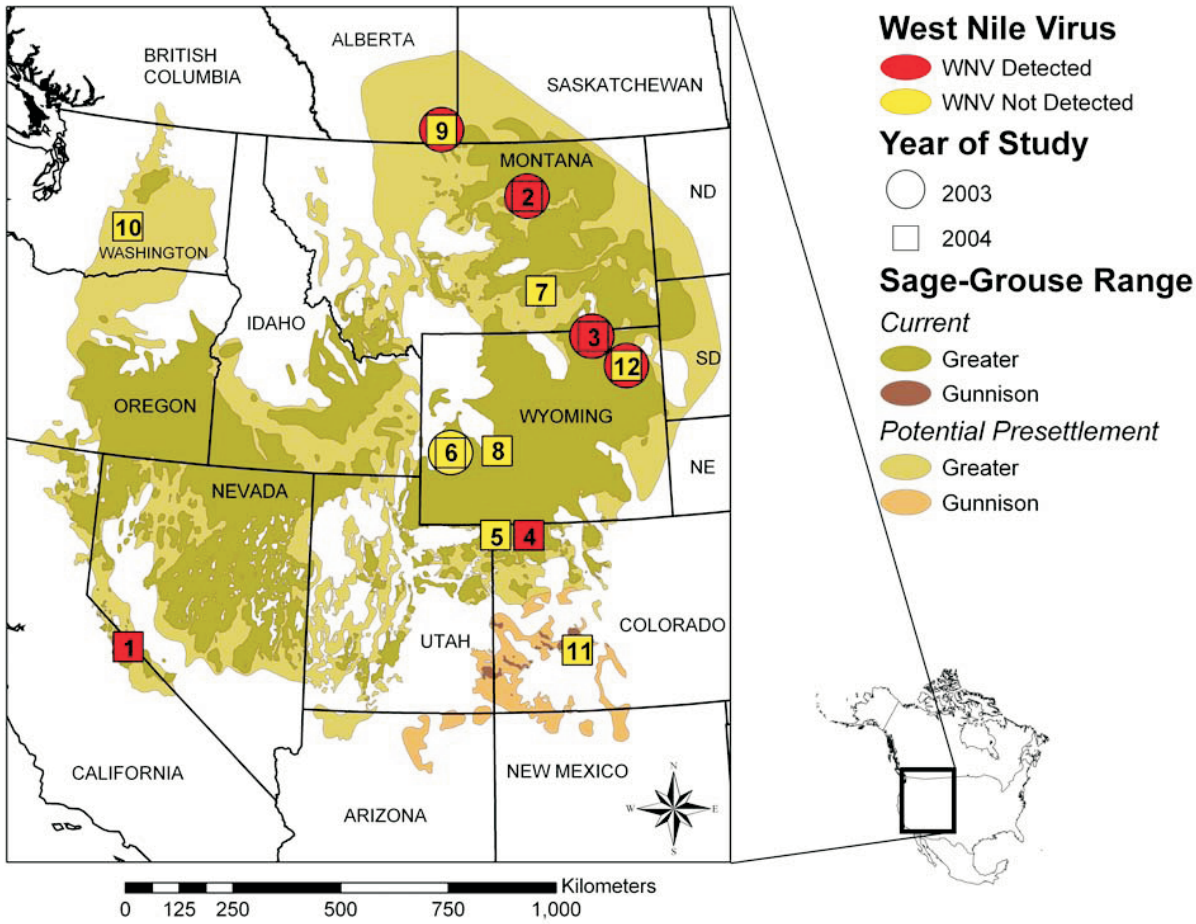

Figure 1. North American distribution of greater sage-grouse (green), Gunnison sage-grouse (tan), and locations where radiomarked birds were monitored for West Nile virus in 2003 and 2004. Numbers correspond with survival rate of radiomarked sage-grouse at the site (Table 1). Sites include Mono County, California (1); Phillips County, Montana (2); Northern Powder River Basin in Wyoming (3); Routt (4) and Moffat (5) counties in Colorado; Upper Green River Basin in Wyoming (6); Roundup, Montana (7); Lander, Wyoming (8); Alberta, Canada (9); Central Washington (10); Gunnison County, Colorado (11); and the Southern Powder River Basin in Wyoming (12). Range map modified from Schroeder et al. (2004).

Testing live birds for exposure to WNv

Serum from 112 live or hunter-harvested sage-grouse collected after the initial outbreak in 2003 showed that none had antibodies (Naugle et al. 2004). In 2004 we continued to search for evidence of resistance to WNv (i.e., antibody production) by collecting serum from an additional 251 live birds from the northern Powder River Basin in Wyoming and Montana, from Phillips County, Montana, and from southeast Alberta where radiomarked individuals were known to have died of WNv. We performed plaque reduction neutralization assays on serum or plasma samples from live-sampled birds and hunter-harvested birds (Weingartl et al. 2003).

Mean daily temperature and timing of WNv mortality

Temperature is an environmental regulator of development in *C. tarsalis* (Brust 1991). Thus, we quantified mean daily temperature in July and August between years (2003 and 2004) in 3 sites that were impacted by WNv in 2003 (Naugle et al. 2004) to assess if temperature was associated with timing and rate of WNv-related mortality in sage-grouse. Temperature data were provided by the Onefour Agriculture and Agri-food Canada Research Station

Table 1. Survival of yearling and adult female sage-grouse in late summer from 12 sites monitored for West Nile virus (WNv) mortalities in 2004.

Monitoring sites[a]	Number monitored	Number of mortalities	Mortalities tested for WNv	Positive tests	Survival rate (%)
Dead birds that tested positive for WNv					
1 Mono County, California	61	10	6	3	83.6
2 Phillips County, Montana	72	12	3	2	83.3
3 Northern Powder River Basin, Wyoming	110	17	9	4	84.5
4 Routt County, Colorado	13	1	1	1	92.3
Tested dead birds with no indication of WNv					
5 Moffat County, Colorado	36	3	1	0	91.7
6 Upper Green River Basin, Wyoming	73	6	2	0	91.8
7 Roundup, Montana	61	4	3	0	93.4
8 Lander, Wyoming	46	3	2	0	93.5
No mortalities within the monitoring timeframe					
9 Alberta, Canada	19	0			100
10 Central Washington	17	0			100
11 Gunnison County, Colorado	16	0			100
12 Southern Powder River Basin, Wyoming	9	0			100

[a] Site numbers correspond with locations in Figure 1.

within the southeast Alberta study site (AAFC–AAC 2004, unpublished weather data), the Sheridan Weather Station Office (WSO) and airport (AP) within the northern Powder River Basin (Western Regional Climate Center, Desert Research Institute, Reno, Nevada), and the Malta 7 E within the Phillips County, Montana, site (Western Regional Climate Center). We also calculated long-term mean daily temperatures in July and August at these sites as a baseline for comparison.

We compared Julian dates of WNv-related mortality using a Mann-Whitney *U* test to determine whether timing of deaths differed between years. We also compared Julian dates of mortality between years at the 2 sites where WNv deaths were documented both years to evaluate whether timing of deaths coincided with latitudinal differences in the gradient of temperatures that we observed. Lastly, we compared Julian dates of mortality within years to determine whether the shift in relative timing of mortality was consistent between years despite latitudinal differences. We used analyses to assess adequacy of the 1 July–31 August monitoring timeframe originally recommended by Walker et al. (2004).

Vector surveillance

We compared prevalence and infection rates of *C. tarsalis* (Goddard et al. 2002) between years. We conducted surveillance during a 9-week period (1 July–7 September) in 2003 and 2004 in southeast Alberta, a site where radiomarked sage-grouse died of WNv in 2003 (Naugle et al. 2004). We captured host-seeking mosquitoes using standard Centers for Disease Control and Prevention (CDC) traps (BioQuip Products, Inc., Rancho Dominguez, Calif.) baited with carbon dioxide from dry ice and operated without lights. We operated traps at least once per week. We activated traps in early evening, and collected mosquitoes after sunrise the following morning. We collected live adult females, euthanized them by freezing, identified them to species, and stored them in pools of <50. Pools were tested for WNv at the Provincial Laboratory in Calgary, Alberta, Canada, using Nucleic Acid Sequence Based Amplification and Reverse-transcripterase Polymerase Chain Reaction. If pools tested positive using Nucleic Acid Sequence Based Amplicification, a Reverse-transcripterase Polymerase Chain Reaction test (Lanciotti et al. 2000) was conducted to confirm the positive test.

Results

Study sites and survival rates

Monitoring intensity in 12 study sites met criteria for inclusion in analyses (Table 1). We excluded data from sites in Idaho, Utah, and Nevada where marked birds were monitored in late summer but carcasses were not retrieved for WNv testing. Investigators from 12 sites monitored 533 radiomarked yearling and adult female sage-grouse, of which 56 (10%) died during the 2004 WNv monitoring period (Table 1). Of the 27 testable carcasses, 10 (37%) were positive for WNv (Table 1). Four WNv-related mortalities were from the northern Powder River Basin in Wyoming and Montana, 2 from Phillips County, Montana, 1 from Routt

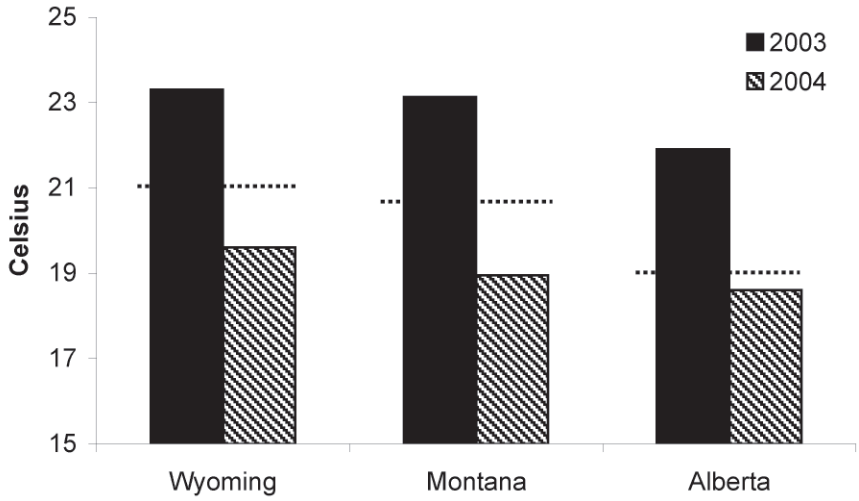

Figure 2. Mean daily temperatures in July and August in Sheridan, Wyoming, Malta, Montana, and southeast Alberta, Canada, during outbreaks in 2003 and in 2004 when fewer birds died. Dashed line indicates long-term average (1948–2004 for Wyoming; 1972–2004 for Montana; 1965–2004 for Alberta).

County, Colorado, and 3 from Mono County, California (Figure 1, Table 1). No late-summer mortalities were reported from 1 monitored population of Gunnison sage-grouse in Colorado. Survival of females in late summer was 6% lower ($P = 0.083$) at 4 sites with confirmed WNv mortalities ($\bar{x}$ = 86% survival, SE = 2.1) than at 4 sites where dead birds were tested but with no indication of WNv ($\bar{x}$ = 92%, SE = 0.5; Table 1). When 4 populations where no individuals died during the monitoring period were included (Table 1), survival of females was 10% lower ($P < 0.016$) at 4 sites with confirmed WNv mortalities ($\bar{x}$ = 86% survival) than at 8 sites without ($\bar{x}$ = 96% survival, SE = 1.4).

Evidence of resistance

Serum collected from all 251 sage-grouse in 2004 tested negative for antibodies. Lack of a positive test from samples across years in 2003 and 2004 (n = 363) suggested that this species currently has no resistance to WNv.

Timing of WNv mortality

Median date of mortality attributable to WNv was 22 days later ($P = 0.01$) in 2004 (31 August) than 2003 (9 August). Half of the mortalities attributable to WNv (5/10) occurred after 31 August in 2004, whereas all WNv-related deaths (16/16) in 2003 were before 27 August. The earlier and higher rate of mortality in 2003 coincided with mean daily temperatures that were 3ºC above the long-term average (Figure 2). The later and lower rate of mortality in 2004 corresponded with temperatures that were 3–4ºC below those in 2003 and ~2ºC below long-term averages for sites in Wyoming, Montana, and Alberta (Figure 2).

No mortality was observed in 2004 in southeast Alberta, the northernmost study site where median date of mortality attributable to WNv mortality in 2003 was 23 August. At the 2 sites where WNv deaths were documented in both years, median date of mortality in 2003 was 17 days later (20 August) in Phillips County, Montana, than in the northern Powder River Basin (3 August; $P = 0.008$), suggesting that timing of mortality also was related to latitude (Figure 2). Despite latitudinal differences, the shift to later timing of mortality was consistent between years in Phillips County, Montana (20 August in 2003 versus 10 September in 2004; $P = 0.064$), and in the northern Powder River Basin (3 August in 2003 versus 24 August in 2004; $P = 0.057$).

Vector surveillance data

The average number of *C. tarsalis* captured per trap night in southeast Alberta declined from 13.4 in 2003 to 3.1 in 2004 (Table 2). Mosquito infection rates also declined from 12.2% in 2003 to <1% in 2004 (Table 2).

Table 2. Abundance of the mosquito *Culex tarsalis* and West Nile virus (WNv) infection rates in southern Alberta, Canada, 2003 and 2004.

	Year	
	2003	2004
Number of trap stations	12	22
Number of *C. tarsalis* captured per trap night	13.4	3.1
Number of pools tested	180	226
Prevalence of WNv per pool	12.2%	0.4%

Table 3. Number of West Nile virus cases reported in humans and horses in Montana, Wyoming, Colorado, California, and Alberta during 2003 and 2004.

	Year	
Cases reported	2003	2004
Montana		
Humans[a]	61	10
Human deaths[a]	4	0
Horses[b]	194	11
Wyoming		
Humans[a]	375	10
Human deaths[a]	9	0
Horses[b]	260	32
Colorado		
Humans[a]	2,947	276
Human deaths[a]	63	3
Horses[b]	426	31
California		
Humans[a]	3	771
Human deaths[a]	0	23
Horses[b]	1	536
Alberta[c]		
Humans	275	0
Human deaths	0	0
Horses	170	4

[a] Data from the Centers for Disease Control and Prevention http://www.cdc.gov/ncidod/dvbid/westnile/ [Date accessed 1 March 2005].

[b] Data from the United States Department of Agriculture. (http://www.aphis.usda.gov/vs/nahps/equine/wnv/ [Date accessed 13 April 2005]).

[c] Data from the Government of Alberta (http://www3.gov.ab.ca/srd/fw/diseases/WNv/ [Date accessed 10 March 2005]).

Discussion

In 2004 WNv decreased survival at 2 sites in Wyoming and Montana that also reported WNv-related mortalities in 2003 (Naugle et al. 2004) and at 2 sites in Colorado and California at which WNv in sage-grouse had not been documented previously. Despite continued vigilance in testing, we still have no evidence that sage-grouse show resistance to WNv. Antibody development may be low in species that are susceptible to WNv; only 3% (5/156) of individuals tested were resistant to infection in an American crow population that lost 68% of its marked individuals to WNv in 2002 (Yaremych et al. 2004). Finding WNv in an isolated population that warrants special protection (i.e., Mono County, California; Oyler-McCance et al. [2005]) is cause for concern because female survival has been identified as a limiting factor in population growth (Johnson and Braun 1999) and losses come at a time of year when survival typically is high (Braun 1998, Schroeder et al. 1999, Connelly et al. 2000*a*, Aldridge and Brigham 2003). Sensitivity analysis (Wisdom et al. 2000) would help to anticipate consequences of WNv on long-term population viability.

The 2004 WNv season was not the catastrophe that many had predicted, and the decrease in prevalence of infection and mortality in sage-grouse, humans, and horses (except California; Table 3) has left many wondering whether the worst has past or if additional outbreaks will occur. Although we cannot yet answer this question, evidence suggests that risk of infection in sage-grouse was low in 2004 because unseasonably cool summer temperatures delayed or reduced mosquito production. Lower incidence of WNv in 2004 corresponded with temperatures that remained well below 21ºC, the threshold temperature below which development in *C. tarsalis* is greatly reduced (Brust 1991). Moreover, timing of WNv mortality was 2–3 weeks later in 2004 than in 2003, and the shift to later timing of mortality was consistent between years despite latitudinal differences at the 2 sites where WNv reduced survival both years. Lastly, mosquito surveillance data indicated a sharp decline in prevalence and infection rate of adult *C. tarsalis* in southeast Alberta, the most northern latitude where WNv reduced survival in 2003 but not in 2004.

The short history of WNv in North America and a poor understanding of factors that lead to an outbreak make predicting future impacts of this disease challenging. Different species exhibit different immune responses (Komar et al. 2003), and individuals that survived infection in 2003 may act as reservoir hosts for WNv. Unfortunately, putative reservoir hosts for WNv in western North America remain unknown. More work on host–vector interactions is needed to understand WNv in sagebrush habitats of western North America.

Management implications

Full understanding of the implications of WNv for sage-grouse populations requires a long-term, coordinated monitoring strategy. This strategy will require an infusion of financial support because monitoring should continue after the nesting and brood-rearing periods. Timing of early mortalities and trends in late-summer temperature also may be useful in gauging the intensity and duration of monitoring needed to quantify impacts of WNv. For example, 50% of WNv mortalities in 2004 occurred

1–17 September, well after the end of the monitoring period suggested in Walker et al. (2004). Thus, we recommend that monitoring continue until the end of September in years when July–August temperatures are below average or for 8–10 days after a hard frost eliminates adult mosquitoes.

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David E. Naugle is an associate professor in the Wildlife Biology Program at the University of Montana (UM) in Missoula. Dave and his lab first found WNv in sage-grouse while investigating potential impacts of coal-bed natural gas development on populations of greater sage-grouse. He completed his M.S. (1994) and Ph.D. (1998) at South Dakota State University. Dave is a past treasurer for the South Dakota Chapter of The Wildlife Society (TWS) and is the current academic advisor for the Student Chapter of TWS at UM. ***Cameron L. Aldridge*** has been studying sage-grouse ecology in Alberta for the past 9 years. He obtained his M.Sc. in biology from the University of Regina and his Ph.D. at the University of Alberta where he assessed the population viability of sage-grouse in Alberta. In spring of 2005, he began a postdoctoral research position with the United States Geological Survey at Colorado State University, addressing sagebrush-steppe conservation. His research interests include population ecology and wildlife management of vertebrates, conservation biology, and endangered species management. He has been a member of TWS since 1999 and is a past executive member of the Alberta Student Chapter of TWS. ***Brett L. Walker*** is a doctoral student in the Wildlife Biology Program at UM in Missoula. He currently is studying population responses of greater sage-grouse to coal-bed natural gas development in the northern Powder River Basin. Brett completed his B.A. (1990) in environmental sciences at the University of California at Berkeley and his M.S. (2000) in avian ecology at UM. For the past 12 years he has studied diverse aspects of songbird and shorebird biology throughout the United States, Canada, and the Caribbean. His professional research interests include the conservation and management of shrub-steppe ecosystems, the evolution of mating, parental care, and communication systems in birds, and the impacts of exotic plants on native wildlife. Brett has been a member of TWS since 2000. ***Kevin E. Doherty*** also is a doctoral student in the Wildlife Biology Program at UM. Kevin is linking greater sage-grouse habitat and population data with satellite imagery to create planning tools that prioritize landscapes for sage-grouse conservation in the Powder River Basin. Kevin graduated with a B.S. in wildlife science from Virginia Tech (1997) and an M.S. in wildlife conservation from the University of Minnesota (2004). ***Marc R. (Randy) Matchett*** is the senior wildlife biologist for the United States Fish and Wildlife Service on the Charles M. Russell National Wildlife Refuge (CMR), where he has been employed since 1987. He has worked for a variety of state and federal agencies in Montana and Idaho on many different wildlife species and issues. Beginning with research on moose in northwestern Montana in 1981, Randy's career has been continually downsizing from work on the big-game species of CMR, to prairie dogs and black-footed ferrets, to fleas and plague bacteria, to sage-grouse and WNv interactions, and most recently to chronic wasting disease surveillance and prions. Most of Randy's time is devoted to prairie wildlife research, management, and habitat conservation efforts. Coordination among the many varied interests and values presents many challenges. Wife Sally and children Sam and Maggie are Randy's constant reminders of the importance of the work of wildlife professionals, and of the importance of family to wildlife professionals. ***Jock McIntosh*** is a biologist and pesticide specialist with Alberta Environment. He administers provincial pesticide legislation and develops pesticide policies, standards, and programs, and provides pesticide consultation services to a variety of provincial and federal agencies. He is a graduate of the University of Calgary and a member of the Alberta Society of Professional Biologists. His interest in environmental science and regulation spans 20 years and includes application, research, education and regulatory experience. Currently, Jock is involved in the development and implementation of mosquito surveillance and control strategies for the Alberta West Nile Virus Response Plan. ***Todd E. Cornish*** is a veterinary pathologist and assistant professor at the Wyoming State Veterinary Laboratory, University of Wyoming in Laramie. He received his B.S. and DVM degrees from the University of California (Davis), a DACVP from North Carolina State University, and a Ph.D. in veterinary pathology from the University of Georgia in Athens. Todd is the consulting veterinarian for field studies involving WNv and sage-grouse, and is principal investigator on a companion project where wild sage-grouse have been brought into captivity to evaluate WNv transmission dynamics. ***Mark S. Boyce*** is professor of biological sciences and the Alberta Conservation Association Chair in Fisheries and Wildlife at the University of Alberta. Mark was Editor-in-Chief of *The Journal of Wildlife Management* 1996–1997; is past president of the Yale University, Wisconsin, and Alberta Chapters of TWS; and serves as the academic advisor for the University of Alberta Student Chapter of TWS.

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