Ecology Letters, (2004) **7**: 704–713 doi: 10.1111/j.1461-0248.2004.00631.x

REPORT

West Nile virus: pending crisis for greater sage-grouse

David E. Naugle,[1]* Cameron L. Aldridge,[2] Brett L. Walker,[1] Todd E. Cornish,[3] Brendan J. Moynahan,[4] Matt J. Holloran,[5] Kimberly Brown,[6] Gregory D. Johnson,[7] Edward T. Schmidtmann,[8] Richard T. Mayer,[8] Cecilia Y. Kato,[8] Marc R. Matchett,[9] Thomas J. Christiansen,[10] Walter E. Cook,[11] Terry Creekmore,[12] Roxanne D. Falise,[13] E. Thomas Rinkes[14] and Mark S. Boyce[2]

Abstract

Scientists have feared that emerging infectious diseases could complicate efforts to conserve rare and endangered species, but quantifying impacts has proven difficult until now. We report unexpected impacts of West Nile virus (WNv) on radio-marked greater sage-grouse (*Centrocercus urophasianus*), a species that has declined 45–80% and is endangered in Canada and under current consideration for federal listing in the US. We show that WNv reduced late-summer survival an average of 25% in four radio-marked populations in the western US and Canada. Serum from 112 sage-grouse collected after the outbreak show that none had antibodies, suggesting that they lack resistance. The spread of WNv represents a significant new stressor on sage-grouse and probably other at-risk species. While managing habitat might lessen its impact on sage-grouse populations, WNv has left wildlife and public health officials scrambling to address surface water and vector control issues in western North America.

Keywords

Centrocercus urophasianus, emerging infectious disease, endangered species, greater sage-grouse, mosquito, population decline, survival, vector surveillance, West Nile virus.

Ecology Letters (2004) 7: 704–713

INTRODUCTION

Emerging infectious diseases present a challenge in the conservation of sensitive or declining wildlife species (McCallum & Dobson 1994; Daszak *et al.* 2000). Since its introduction to New York in 1999, West Nile virus (WNv) has rapidly spread west across North America, infecting and killing wild and domestic birds, horses, humans, and other animals (Centers for Disease Control and Prevention, 2004). At least 228 species of birds, 29 mammals, and two reptiles have been infected with WNv (Centers for Disease Control and Prevention, 2004). The virus has reached 44 US states, seven Canadian provinces, Mexico, and the Caribbean, and continues to spread (Estrada-Franco *et al.* 2003). Although many bird species are susceptible, the impact of WNv on native, wild bird populations is virtually unknown (Marra *et al.* 2004). Data from wild avian populations are limited to two studies of the common and abundant American crow

[1]*Wildlife Biology Program, College of Forestry and Conservation, University of Montana, Missoula, MT 59812, USA*
[2]*Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9*
[3]*Wyoming State Veterinary Laboratory, Department of Veterinary Sciences, University of Wyoming, Laramie, WY 82070, USA*
[4]*Boone and Crockett Wildlife Conservation Program, College of Forestry and Conservation, University of Montana, Missoula, MT 59812, USA*
[5]*Wyoming Cooperative Fish and Wildlife Research Unit, University of Wyoming, Laramie, WY 82070, USA*
[6]*Thunderbird Wildlife Consulting, Inc., Wright, WY 82732, USA*
[7]*Department of Entomology, Montana State University, Bozeman, MT 59717, USA*
[8]*USDA-ARS, Arthropod-borne Animal Diseases Research Laboratory, University of Wyoming, Laramie, WY 82071 USA*
[9]*US Fish and Wildlife Service, Charles M. Russell National Wildlife Refuge, Lewistown, MT 59457, USA*
[10]*Wyoming Game and Fish Department, Green River, WY 82935, USA*
[11]*Wyoming Game and Fish Department, Laramie, WY 82070, USA*
[12]*Department of Health, Laramie, WY 82070, USA*
[13]*Bureau of Land Management, Billings, MT 59107, USA*
[14]*Bureau of Land Management, Lander, WY 82520, USA*
**Correspondence: E-mail: dnaugle@forestry.umt.edu*

(*Corvus brachyrhynchos* Brehm), in which mortality of marked individuals was 40–68% (Caffrey *et al.* 2003; Yaremych *et al.* 2004). The impacts of WNv may be more severe for species already threatened by habitat loss and for those in small, isolated populations, particularly if they show high susceptibility to the virus.

Greater sage-grouse (*Centrocercus urophasianus* Bonaparte; hereafter 'sage-grouse'; Fig. 1) is native to sagebrush habitats of western North America. Previously widespread, the species has been extirpated from much of its original range, with an estimated range-wide population decline of 45–80% and local declines of 17–92% (Braun 1998; Connelly *et al.* 2000b; Aldridge & Brigham 2003). Loss and degradation of suitable nesting and brood-rearing habitats from anthropogenic change was thought, until now, to be the single most important factor leading to fragmentation, reduction, and extirpation of populations (Braun 1998; Connelly *et al.* 2000a,b; Aldridge & Brigham 2002; Knick *et al.* 2003).

We document declines in late-summer survival in radio-marked adult female sage-grouse caused by infection with WNv in their eastern range (Fig. 2). This work resulted from a rapid, coordinated effort between US and Canadian biologists and land managers after discovery of the first case of WNv-caused mortality of a sage-grouse on 24 July 2003.

METHODS

We monitored radio-marked sage-grouse from March to September in: (1) southeastern Alberta, (2) southern Phillips County (SPC), Montana, (3) the northern Powder River Basin (NPRB) of southeastern Montana and northeastern Wyoming (north of Interstate-90), (4) the southern Powder River Basin (SPRB) in northeastern Wyoming, and (5) the upper Green River Basin (UGRB) in western Wyoming (Fig. 3). During July and August from 1998 to 2002 for study sites 1–4, we detected 25 deaths among 284 (8.8%) radio-collared adult females. During July and August 2003, a total of 56 mortalities were detected among 242 (23.1%) radio-collared females from five study sites.

Dead sage-grouse were tested for WNv at the Wyoming State Veterinary Laboratory (birds from WY and MT) or at the Canadian Cooperative Wildlife Heath Center (birds from Alberta) in Saskatoon, Saskatchewan. All sage-grouse underwent complete necropsies, microscopic examination of routine tissues by histopathology, and were tested for WNv using two tests, real time polymerase chain reaction (RT-PCR; Shi 2001) and immunohistochemistry (Kiupel *et al.* 2003). Select cases positive for WNv were confirmed by isolation of the virus from several tissues (brain, heart, and kidney) in Vero cell cultures (Steele *et al.* 2000).

We estimated late-summer survival of radio-marked adult female sage-grouse (≥1 year old) using monitoring data from the five marked populations. We define late summer as the 2-month period from 1 July to 31 August that coincides with the peak season for WNv exposure in humans and wildlife in this region. We detected mortalities using mortality sensors on radio-collars or by visually confirming the status of each individual at regular intervals (typically every 2–7 days). Because carcasses do not always yield testable samples, survival estimates based strictly on recovered carcasses are likely to underestimate mortalities due to WNv infection. Moreover, predators may preferentially capture and consume infected birds, and

Figure 1 Male greater sage-grouse displaying on a lek (photo: Gordon Court).

Figure 2 Radio marked adult female greater sage-grouse (photo: Steve Slater).

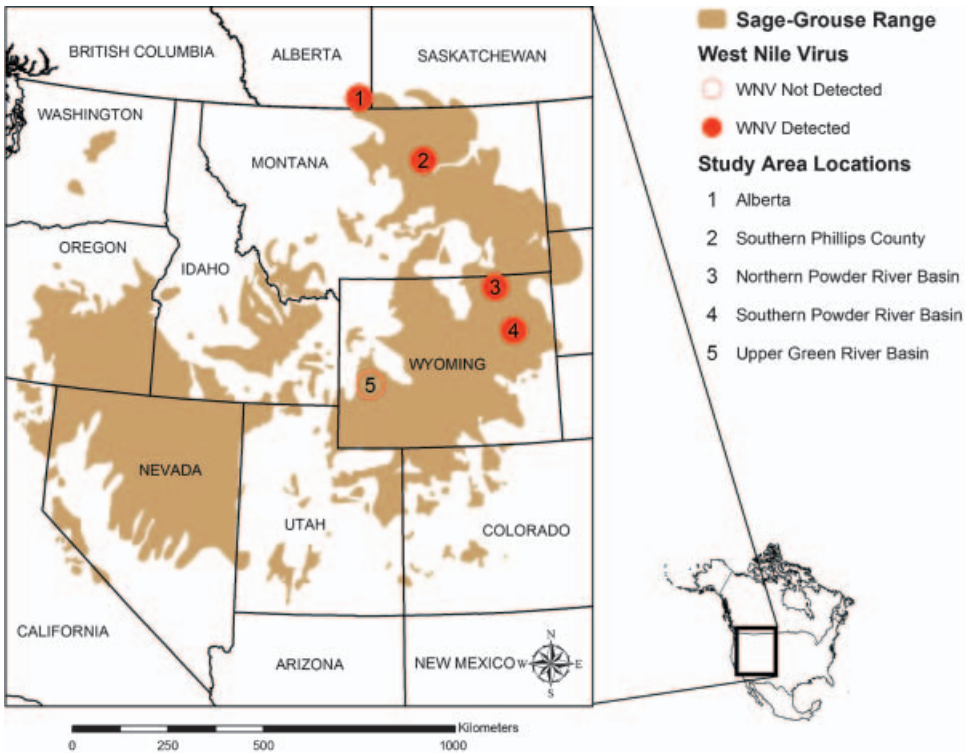

Figure 3 North American distribution of greater sage-grouse (tan) and locations of five study sites. Range map provided by Michael A. Schroeder, Washington Department of Fish and Wildlife (http://sagemap.wr.usgs.gov/).

carcasses may decompose or be scavenged prior to recovery. To estimate the impact of WNv on survival, we compared background survival rates among years with and without WNv and among affected and unaffected sites in 2003 (Fig. 3). The NPRB represents a new study site in 2003 that was not monitored pre-WNv emergence.

Survival estimation

We used a Kaplan–Meier product limit estimator (Kaplan & Meier 1958) with a staggered entry design (Winterstein *et al.* 2001) in STATA 7.0 (STATA Corporation, College Station, TX, USA; STATA 2001) to estimate late-summer survival. Each year, new individuals [yearlings and adults (>1 year of age)] were captured and fixed with radio-collars at each site to maintain numbers of marked birds. We used a log-rank test (chi-square statistic) to test for differences in survival functions (Winterstein *et al.* 2001) between study areas, and with respect to the detection of WNv; both within study areas and for combined data. We then used the Andersen–Gill (A–G) version of the Cox proportional hazards model to test for differences in survival functions (Andersen & Gill 1982). The A–G approach allowed us to accommodate left- and right-censoring and discontinuous intervals of risk in our monitoring data (Andersen & Gill 1982). When sage-grouse tracking sessions were >14 days apart, or individuals were lost prior to the end of the August tracking period (i.e. fate unknown) they were right-censored from the dataset on the last date they were located. We estimated dates of mortality as the mid-point between last date observed alive and the first date observed dead. In some cases, date of death was estimated more accurately based on condition of the carcass (e.g. fresh or decomposed) and temperature during the interval between observations.

The principle assumption when comparing survival functions with A–G Cox model is that the hazards are proportional over time (Andersen & Gill 1982; Winterstein *et al.* 2001) or the influence of a treatment or independent variable on the risk of death does not change over the duration of the study (Johnson *et al.,* in press). We compared plots of the logarithms of the estimated cumulative hazard functions (Andersen & Gill 1982; Cleves *et al.* 2004) and Schoenfeld residuals (Schoenfeld 1982) to test for violations of the proportional hazards assumption. If logarithm plots with curves of treatment groups are not parallel or plots of Schoenfeld residuals indicate a non-zero slope, the proportional hazards assumption is violated, preventing comparison of survival curves using the A–G Cox approach. We used a chi-square goodness of fit test on the Schoenfeld residuals to statistically test for a non-zero slope (Grambsch & Therneau 1994; Cleves *et al.* 2004).

We developed candidate A–G models using categorical variables to test for the impact of WNv on greater sage-grouse survival and for differences between study sites. For models containing study site as a categorical variable, the UGRB (control site) was used as the reference category (indicator contrasts) for comparison of study sites. We used the Breslow estimation of the continuous-time likelihood calculation to partition deaths with tied failure times (Cleves *et al.* 2004). We took an information theoretic approach using Akaike Information Criteria (AIC) for model selection (Anderson *et al.* 2000). Cox Regression proportional hazards models are a semi-parametric approach, in which the baseline hazard is not parameterized and is left unestimated (Cleves *et al.* 2004). The intercept (β_0) is included in the baseline hazard and therefore not estimated, excluding it as an estimated parameter from AIC calculations. We used the differences in AIC scores (Δ_i) to identify the best approximating model (i.e. most explanatory power) within the set (Anderson *et al.* 2000). AIC weights (w_i) were used to assess the approximate probability that each model was the best model of the given set (Anderson *et al.* 2000). We used a likelihood ratio chi-square statistic to test the significance of the best approximating model and we assessed the effect of each parameter on survival using 95% confidence intervals; intervals that did not overlap zero contributed to the survival model. We used exponentiated linear coefficients from the A–G model to generate risk ratios (Winterstein *et al.* 2001; Cleves *et al.* 2004) and assess the risk of death relative to each parameter (WNv or study site).

WNv monitoring in sage-grouse

To test whether birds that survived had been exposed to WNv, we collected serum from 41 live birds captured from within the NPRB study site between 28 August and 25 September 2003, and from six females in the southeastern Alberta site in September 2003. We also analyzed serum and tissue samples from 65 sage-grouse harvested throughout Wyoming in September 2003 that were collected by the Wyoming Game and Fish Department and the Bureau of Land Management. Plaque reduction neutralization assays were performed on serum or plasma samples from live-sampled birds and hunter-harvested birds (Weingartl *et al.* 2003).

Vector monitoring

Vector monitoring was initiated in the NPRB study site after the first sage-grouse mortality was discovered there to identify primary arthropod vectors of WNv and to estimate their infection rates. Trapping was conducted in the NPRB study site between 18 August and 22 September 2003. We used standard arbovirus surveillance methods (United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention (CDC),

National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases 2003) to capture mosquitoes and biting midges in miniature black light/suction traps (John W. Hock, CO., Gainsville, FL, USA) that were baited with 1 kg dry ice and set adjacent to aquatic habitats at sunset. Trap catch bags were collected the following morning and held in a chilled ice chest prior to insect identification and processing. Mosquitoes and biting midges were sorted to species after being quick-frozen on dry ice. Specimens were stored at −80 °C on liquid nitrogen before RNA extraction.

RNA extraction was performed on 101 pools of female *Culex tarsalis* (Coquillett) (20 specimens maximum per pool; 1774 individuals) and on 19 pools of *Culicoides sonorensis* (Wirth and Jones) (50 specimens maximum per pool; 903 individuals) (Table 3) with the RNeasy 96 kit (Qiagen, Valencia, CA, USA). Samples were ground in liquid nitrogen, mixed with 1 mL Buffer RLT and centrifuged at 8000 × ***g*** for 10 min. Half of the supernatant was stored at −80 °C, and the remaining was used in the extraction according to manufacturer's specifications. Approximately 50 μL of eluate was recovered per sample, and stored at −20 °C until used in the TaqMan assay. RT-PCR was run (Lanciotti *et al.* 2000) on the ABI Prism 7000 Sequence Detection System with TaqMan One Step RT-PCR master mix reagents (Applied Biosystems, Foster City, CA, USA). Primer and probe combinations (DNA Technologies Inc., Coralville, IA, USA) were then synthesized (Lanciotti *et al.* 2000; Lanciotti & Kerst 2001). Positive samples from the WNENV primer/probe were then tested with the WN3'NC primer/probe set. Pools were considered positive when CT values were <37, and the normalized fluorescent signal (Rn) was 2 × greater than the average of eight non-template controls for both primer/probe sets. Infection rates were calculated, adjusting for sample sizes (Biggerstaff 2003).

RESULTS

Mortality caused by WNv infection occurred among radio-marked female sage-grouse from four study sites (Alberta, SPC, NPRB, and SPRB) between 1 July and 31 August 2003 (Fig. 3). Of 22 testable carcasses, WNv was confirmed to be the cause of death in 18 cases: 5/5 cases in southeastern Alberta, including two juveniles; 4/4 cases in SPC, Montana; 7/11 cases in the NPRB, including one male; and 1/2 cases in the SPRB of northern Wyoming. Although small sample sizes precluded further analysis, WNv-induced deaths of one male and two chicks (*ca.* 10 weeks old) suggest that the virus does not discriminate between sex and age classes. Five additional unmarked dead sage-grouse from outside our study sites also tested positive for WNv in the following Wyoming counties: Big Horn, Carbon, Fremont, Natrona, and Sweetwater. No deaths due to WNv were detected in radio-marked females from the UGRB in western Wyoming (i.e. control site).

Testing model assumptions

Prior to 2003, log rank tests indicated that adult female survival over the WNv period was similar between the four

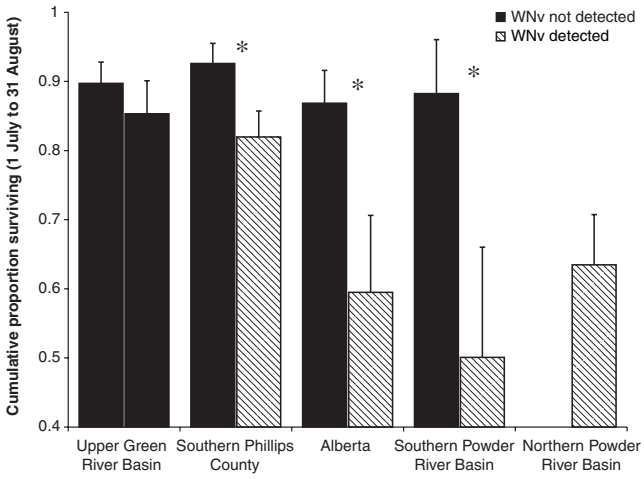

Figure 4 Kaplan–Meier survival estimates for adult female greater sage-grouse during the West Nile virus period (1 July–31 August). Survival is shown for each study before (black) and during (hatched) the West Nile virus (WNv) period in 2003. The upper Green River Basin (UGRB) where WNv was not detected is a control site (black in 2003). The Northern Powder River Basin (NPRB) was not monitored pre-WNv emergence. Number of females monitored within sites in years pre-WNv exposure and in 2003 were: UGRB $n = 110$ in 2000–02, 55 in 2003; the southern Phillips County (SPC) $n = 85$ in 2002, 109 in 2003; Alberta (AB) $n = 71$ in 1998–2002, 23 in 2003; the southern Powder River Basin (SPRB) $n = 18$ in 2001–2002, 11 in 2003; NPRB $n = 44$ in 2003. Asterisks indicate significant differences (log-rank test) in survival within sites (UGRB, $\chi^2_1 = 1.00$, $P = 0.316$; SPC, $\chi^2_1 = 4.55$, $P = 0.033$; AB, $\chi^2_1 = 5.88$, $P = 0.015$; SPRB, $\chi^2_1 = 5.81$, $P = 0.016$).

study sites monitored pre-WNv emergence ($\chi^2_3 = 1.55$, $P = 0.67$; Fig. 4), even though a single confirmed WNv-related death occurred at the SPRB in 2002. Within the WNv year (2003), differences in survival existed between study sites ($\chi^2_4 = 17.01$, $P = 0.002$; Fig. 4), even when the UGRB control site was excluded and only the four studies with WNv are considered ($\chi^2_3 = 14.00$, $P < 0.003$; Fig. 4). Survival for the control site (UGRB) in 2003 also was similar to that for all other sites combined, prior to 2003 ($\chi^2_1 = 1.0$, $P = 0.32$, Fig. 4).

For the four study areas with multiple years of data, we tested the proportional hazards assumption comparing survival for 2003 (WNv year) to survival in previous years (each set of study area bars in Fig. 4). For the three WNv affected study sites, logarithm plots of the estimated hazard functions for the two treatments within each group were roughly parallel, and tests of non-zero slopes of the Schoenfeld residuals were non-significant (SPC: $\chi^2_1 = 0.48$, $P = 0.49$; Alberta: $\chi^2_1 = 2.69$, $P = 0.10$; SPRB: $\chi^2_1 = 0.26$, $P = 0.61$), indicating that data within these three study sites did not violate the proportional hazards assumptions. A test of nonzero slope of the Schoenfeld residuals for the UGRB control site was significant ($\chi^2_1 = 7.85$, $P = 0.005$), indicating a violation of the proportional hazards assumption. However, since WNv was never detected in the UGRB, data from this site in 2003 were pooled with previous years of non-WNv data for all between year analyses.

We tested for the overall effect of WNv on sage-grouse by grouping all data into two treatment groups, WNv detected and WNv not detected (Fig. 4). Logarithm plots of the estimated cumulative hazard functions indicated that curves for the two treatments were parallel, suggesting our combined data did not violate the proportional hazards assumption. A goodness-of-fit test of the non-zero slopes in Schoenfeld residuals was non-significant ($\chi^2_1 = 0.19$, $P = 0.67$), also indicating that data met the proportional hazards assumption. Lastly, we tested for differences in survival within the 2003 WNv year by comparing the UGRB control site where WNv was not detected, to survival of all other four sites combined. Parallel logarithm plots and the slope of the Schoenfeld residuals were not different from zero ($\chi^2_1 = 3.39$, $P = 0.06$) indicating that these data met the proportional hazards assumption.

Comparison among years with and without WNv

Late-summer survival declined significantly (−14.5%) between pre-WNv years (89.6 ± 2.05%, 1998–2002) and 2003 (75.1 ± 2.92%; $\chi^2_1 = 15.77$, $P < 0.001$, all sites grouped; Fig. 4), and more so (−15.8%) when the UGRB in 2003 is considered a non-WNv year (88.7 ± 1.9% for non-WNv; 72.9 ± 3.35% for WNv; $\chi^2_1 = 17.70$, $P < 0.001$, data for UGRB control site in 2003 grouped with all pre-WNv year data; Fig. 4). Within the four WNv affected study sites, averaged survival estimates indicate that late-summer survival in a WNv year (2003; 63.8 ± 6.68%; Fig. 4) decreased by 25.3% compared to survival in pre-WNv years (1998–2002; 89.0 ± 1.25%; Fig. 4).

Comparison among control and WNv sites in 2003

Late-summer survival was significantly lower (−25.9%) at the four sites affected by WNv in 2003 (63.8%) compared

Table 1 Candidate Andersen–Gill multiplicative hazard models, Akaike Information Criteria (AIC) results for effects of West Nile virus (WNv) on survival of adult female greater sage-grouse

Model	K_i	Log-likelihood	AIC	Δ_i	w_i	Rank
WNv + Study site	5	−474.60	959.20	0	0.765	1
WNv + Study site + WNv × Study site	*7	−473.98	961.96	2.76	0.192	2
WNv	1	−481.47	964.94	5.75	0.043	3

Number of parameters (model complexity) is represented by K_i. Models were evaluated based on differences among AIC scores (Δ_i) and AIC weights (w_i). Model rank is based on differences among AIC scores.

*Interaction terms for two study sites (Alberta and NPRB) were removed due to collinearity.

Table 2 Estimated coefficients, standard errors, and 95% confidence intervals for the best Akaike Information Criteria model

			Confidence intervals	
Variable	Coefficient	SE	Upper	Lower
WNv*	1.229	0.327	0.589	1.870
Study Site†				
Alberta	0.027	0.392	−0.741	0.794
Southern Phillips County	−0.891	0.409	−1.692	−0.090
Northern Powder River Basin	−0.105	0.476	−1.038	0.828
Southern Powder River Basin	0.259	0.482	−0.741	0.794

Andersen–Gill proportional hazard model (West Nile virus (WNv) + Study site) describing the impact of WNv on greater sage-grouse. The Upper Green River Basin (UGRB; control site) was used as the reference category (indicator contrasts) for comparison of study sites.

*Positive coefficient indicates increased hazard for WNv detection (1) vs. no WNv (0).

†Positive coefficients indicate increased hazard for that site (1) compared with UGRB control site (0).

with the UGRB control (89.7%, $\chi^2_1 = 14.00$, $P < 0.007$, all sites grouped; Fig. 4).

Andersen–Gill proportional hazards models

We developed three different candidate models using WNv, study site, and interaction terms to predict greater sage-grouse survival (Table 1). The WNv + Study Site model was the best approximating model (smallest Δ_i AIC score, Table 1). The model was significant ($\chi^2_5 = 30.57$, $P < 0.001$) and the Akaike weight (AIC $w_i = 0.765$, Table 1) suggested high probability that the model was the best approximating model within the candidate model set. The confidence intervals for WNv ($\beta = 1.229$) did not overlap zero (0.589–1.870, Table 2), indicating that survival decreased in the presence of WNv. The exponentiated linear coefficient for WNv indicated that sage-grouse are 3.4 [exp(1 × 1.229)] times more likely to die in WNv infected locations compared to pre WNv-exposure or the UGRB control site without WNv.

The Alberta, NPRB and SPRB study sites all had similar survival compared to the UGRB control site (confidence intervals all overlap zero, Table 2). However, the confidence interval for the SPC site did not overlap zero ($\beta = -0.891$, 95% CI: −1.692 to −0.090, Table 2) indicating that survival at the SPC site was elevated compared to the UGRB site (Table 2). The exponentiated linear coefficient indicated that sage-grouse have 0.41 [exp(1 × −0.891)] times the chance of death (reduced risk) in the SPC study compared to the UGRB control site. As a comparison for all sites, we also re-ran this model using each individual study site as the reference category. In all cases, the SPC study site had lower relative risk (β coefficient was negative and confidence intervals did not overlap zero). Similarly, when the SPC site was used as the reference category, all four other study sites showed a higher relative

Table 3 Summary of polymerase chain reaction assay data for West Nile virus (WNv) in *Culex tarsalis* and *Culicoides sonorensis*, Northern Powder River Basin study site, WY, 2003

Location	Pools	Specimens	Pools WNv+	C_T/ΔRn WNvENV, WNv3'NC*
C. tarsalis				
A	8	146	3	18.1/5.1; 21.2/6.1 23.2/3.9; 25.1/2.3 18.8/5.4; 21.4/6.5
B	4	58	1	23.9/3.9; 31.0/0.7
C	25	487	1	29.3/4.3; 34.5/2.1
D	3	38	0	
E	2	32	0	
F	30	587	2	28.4/4.1; 28.6/3.4 27.2/2.3; 27.7/4.4
H	2	21	0	
I	1	6	0	
J	2	27	0	
K	1	4	0	
L	1	12	1	18.7/5.3; 20.1/6.6
M	1	1	0	
N	10	171	2	21.6/5.3; 22.9/6.2 32.0/1.1; 33.9/1.5
O	5	64	0	
Q	6	120	2	30.4/4.1; 29.3/4.6 20.1/3.6; 26.4/6.0
Total	101 (7.16)†	1774	12 (11.9%)	
C. sonorensis				
G	2	100	0	
P	1	3	0	
R	16	800	2	32.0/4.0; 33.0/4.0 29.9/4.3; 33.3/3.8
Total	19 (2.31)†	903	2 (22.2%)	

*TaqMan assay: Interpretation of positive; C_T values of <37, and a normalized fluorescent signal (Rn) of 2 × greater than the average of eight non-template controls for both primers 'WNv-ENV' and 'WNv-3'NC'.
†Infection rate/1000.

risk (β coefficient was positive and confidence intervals did not overlap zero), indicating that the SPC site had a higher overall survival rate.

WNv monitoring in sage-grouse and insects

Serum collected from 112 sage-grouse to test whether birds that survived had been exposed to WNv all tested negative for WNv antibodies. Twelve pools of female *C. tarsalis* (11.9%) tested positive for WNv with an infection rate of 7.16 per 1000 (Table 3). Two pools of female *C. sonorensis* (22.2%) tested positive for WNv with an infection rate of 2.31 per 1000 (Table 3).

DISCUSSION

Two lines of evidence led us to conclude that WNv reduced late-summer survival. Data where sage-grouse were monitored both before WNv (1998–2002) and in 2003 indicate that survival declined an average of 25%, whereas survival did not decline in the UGRB, a site where WNv has not been detected in sage-grouse (Fig. 4). Survival at the four locations with WNv-induced mortality in 2003 was, on average, 26% lower than in the UGRB control site (Fig. 4). Overall, individuals in populations exposed to the virus were 3.4 times more likely to die during the 2-month WNv period than birds in uninfected populations.

If survival in our marked sample is representative of broader impacts of WNv, the virus may be an important new stressor on sage-grouse populations. Survival of adult females has been shown to be limiting in sage-grouse populations (Johnson & Braun 1999) and declines due to WNv occurred in late summer when survival typically is high (Braun 1998; Schroeder *et al.* 1999; Connelly *et al.* 2000a; Aldridge & Brigham 2003).

Of immediate concern are the potential consequences of WNv for small populations of Gunnison sage-grouse (*C. minimus* Bradbury and Vehrencamp) in Colorado and Utah, and greater sage-grouse in California, Utah, Washington, North and South Dakota, Alberta, and Saskatchewan. In small, fragmented populations, stochastic events such as disease exacerbate risk of extinction due to the combined effect of demographic stochasticity, deterministic stressors, and inbreeding depression (Soule & Mills 1998; Westemeier *et al.* 1998). Moreover, because small or isolated populations generally show reduced genetic variation, they are less likely to contain individuals resistant to emerging infectious disease (Acevedo-Whitehouse *et al.* 2003).

Knowing whether sage-grouse survive WNv infection is crucial to anticipating possible long-term effects on populations (Komar *et al.* 2003). In a survey of 112 birds from Alberta, Montana, and Wyoming in fall 2003 from areas with confirmed WNv deaths in sage-grouse, we found no live sage-grouse seropositive for neutralizing antibodies against WNv. Thus, we have no evidence that sage-grouse are able to survive WNv infection and develop immunity. Since no other information exists on sage-grouse susceptibility to WNv, we recently brought 35 wild sage-grouse into captivity at the Wyoming State Veterinary Laboratory that will be challenged with the virus to evaluate transmission dynamics.

Vector surveillance near ponds in the NPRB site indicated that the mosquito *C. tarsalis*, a highly competent vector of WNv that breeds in surface waters of western North America (Reisen & Reeves 1990; Goddard *et al.* 2002), was infected with WNv (Table 3). Detection of WNv in *C. sonorensis* was unexpected; *C. sonorensis* is a biting midge known to transmit viral pathogens of livestock and wild ungulates, but previously has not been recognized as a potential vector of WNv (Mellor *et al.* 2000, Table 3).

Surface water sources in arid western landscapes have been created for agricultural irrigation, drinking access for livestock, and oil and gas activities. sage-grouse thrive in native sagebrush-steppe that is largely devoid of natural sources of standing water; however, females and broods naturally move to water in late summer if it is available (Connelly *et al.* 2000b). Although man-made water sources that attract sage-grouse also expose them to insects that vector WNv, the basic biology of arthropod vectors suspected of transmitting WNV in western North America is poorly understood. Thus, in 2004 we have linked vector surveillance in Montana and Wyoming to specific surface water sources to quantify their relative contribution to vector production.

The emergence of WNv further complicates the difficult task of conserving sage-grouse in western North America. Efficacy of mosquito control with pesticides over vast areas of sage-grouse range remains untested, and the suggestion of land-use change only fuels conflict over water management in the west. Petitions to list sage-grouse under the federal Endangered Species Act are intended to force decisions on issues that could change the management of public and private lands. Regardless, if we are to prevent sage-grouse from going extinct on their remaining range, we must find a way to provide high-quality habitats that support robust, genetically diverse populations capable of withstanding stochastic disease events.

ACKNOWLEDGEMENTS

Critical reviews by L. Scott Mills, Roman Biek and four anonymous reviewers improved our manuscript. We thank each author's member institution for funding this research. Additional project support came from the Bureau of Land Management (Washington, DC), Montana Department of Fish, Wildlife and Parks, National Fish and Wildlife Foundation, National Science Foundation (EPS-CORE program),

Montana Cooperative Wildlife Research Unit, Petroleum Association of Wyoming, Wolf Creek Charitable Foundation, Anheuser-Busch Companies, Inc., Alberta Conservation Association, Alberta Sport, Recreation, Parks and Wildlife Foundation, Alberta Sustainable Resource Development, Canadian Wildlife Foundation, Ducks Unlimited-Canada (NAWMP), Endangered Species Recovery Fund, the Natural Sciences and Engineering Research Council of Canada, and the Izaak Walton Killam Memorial Scholarship Fund.

REFERENCES

Acevedo-Whitehouse, K., Gulland, F., Greig, D. & Amos, W. (2003). Disease susceptibility in California sea lions. *Nature*, 422, 35.

Aldridge, C.L. & Brigham, R.M. (2002). Sage-grouse nesting and brood habitat use in southern Canada. *J. Wildl. Manag.*, 66, 433–444.

Aldridge, C.L. & Brigham, R.M. (2003). Distribution, status and abundance of Greater Sage-Grouse, *Centrocercus urophasianus*, in Canada. *Can. Field Nat.* 117, 25–34.

Andersen, P.K. & Gill, R.D. (1982). Cox's regression model for counting processes: a large sample study. *Ann. Stat.*, 10, 1100–1120.

Anderson, D.R., Burnham, K.P. & Thompson, W.L. (2000). Null hypothesis testing: problems, prevalence, and an alternative. *J. Wildl. Manag.*, 64, 912–923.

Biggerstaff, B.J. (2003). *PooledInfRate: A Microsoft*® Excel Add-In to Compute Prevalence Estimates from Pooled Samples. Centers for Disease Control and Prevention, Fort Collins, CO, USA. Available from: http://www.cdc.gov/ncidod/dvbid/westnile/software.htm; modified on 6 April 2004.

Braun, C.E. (1998). sage-grouse declines in western North America: what are the problems? *Proc. West. Assoc. State Fish Wildl. Agencies*, 78, 139–156.

Caffrey, C., Weston, T.J. & Smith, S.C.R. (2003). High mortality among marked crows subsequent to the arrival of West Nile virus. *Wildl. Soc. Bull.*, 31, 870–872.

Centers for Disease Control and Prevention (2004). *Maps of West Nile virus activity*. Available from: http://www.cdc.gov/ncidod/dvbid/westnile/index.htm; modified on 31 March 2004.

Cleves, M.A., Gould, W.W. & Gutierrez, R.G. (2004). *An Introduction to Survival Analyses Using* STATA. Stata Press, College Station, TX, USA.

Connelly, J.W., Apa, A.D., Smith, R.B. & Reese, K.P. (2000a). Effects of predation and hunting on adult sage-grouse *Centrocercus urophasianus* in Idaho. *Wildl. Biol.* 6, 227–232.

Connelly, J.W., Schroeder, M.A., Sands, A.R. & Braun, C.E. (2000b). Guidelines to manage sage-grouse populations and their habitats. *Wildl. Soc. Bull.*, 28, 967–985.

Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2000). Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science*, 287, 443–449.

Estrada-Franco, J.G., Navarro-Lopez, R., Beasley, D.W.C., Coffey, L., Carrara, A., da Rosa, A.T. *et al.* (2003). West Nile virus in Mexico: evidence of widespread circulation since July 2002. *Emerg. Infect. Dis.*, 9, 1604–1607.

Goddard, L.B., Roth, A.E., Reisen, W.K. & Scott, T.W. (2002). Vector competence of California mosquitoes for West Nile virus. *Emerg. Infect. Dis.*, 8, 1385–1391.

Grambsch, P.M. & Therneau, T.M. (1994). Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*, 81, 515–526.

Johnson, K.H. & Braun, C.E. (1999). Viability of an exploited sage-grouse population. *Conserv. Biol.*, 13, 77–84.

Johnson, C.J., Boyce, M.S., Schwartz, C.C. & Haroldson, M.A. (in press). Modelling survival: application of the Anderson–Gill model to Yellowstone grizzly bear. *J. Wildl Manag.*

Kaplan, E.L. & Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.*, 53, 457–481.

Kiupel, M.H., Simmons, H.A., Fitzgerald, S.D., Wise, A., Sikarskie, J.G., Cooley, T.M. *et al.* (2003). West Nile virus infection in eastern fox squirrels (*Sciurus niger*). *Vet. Pathol.*, 40, 703–707.

Knick, S.T., Dobkin, D.S., Rotenberry, J.T., Schroeder, M.A., Van der Haegen, W.M. & Van Riper, C. Jr (2003). Teetering on the edge of too late? Conservation and research issues for avifauna of sagebrush habitats. *Condor*, 105, 611–634.

Komar, N., Langevin, S., Hinten, S., Nemeth, N., Edwards, E., Hettler, D. *et al.* (2003). Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg. Infect. Dis.*, 9, 311–322.

Lanciotti, R.S. & Kerst, A.J. (2001). Nucleic acid sequence-based amplification assays for rapid detection of West Nile and St. Louis encephalitis viruses. *J. Clin. Microbiol.*, 39, 4506–4513.

Lanciotti, R.S., Kerst, A.J., Nasci, R.S., Godsey, M.S., Mitchell, C.J., Savage, H.M. *et al.* (2000). Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J. Clin. Microbiol.*, 38, 4066–4071.

Marra, P.P, Griffing, S., Caffrey, C., Kilpatrick, A.M., McLean, R., Brand, C. *et al.* (2004). West Nile virus and wildlife. *BioScience*, 54, 393–402.

McCallum, H. & Dobson, A. (1994). Detecting disease and parasite threats to endangered species and ecosystems. *TREE*, 10, 190–194.

Mellor, P.S., Boorman, J. & Baylis., M. (2000) *Culicoides* biting midges: their role as arbovirus vectors. *Annu. Rev. Entomol.*, 45, 307–340.

Reisen, W.K. & Reeves, W.C. (1990). Bionomics and ecology of *Culex tarsalis* and other potential mosquito vector species. In: *Epidemiology and Control of Mosquito-Borne Arboviruses in California, 1943–1987* (ed. Reeves, W.C.). California Mosquito and Vector Control Association, Sacramento, pp. 254–329.

Schoenfeld, D. (1982). Partial residuals for the proportional hazards regression model. *Biometrika*, 69, 239–241.

Schroeder, M.A., Young, J.R. & Braun, C.E. (1999). Sage Grouse (*Centrocercus urophasianus*). In: *Birds of North America, No. 425* (eds Pool, A. & Gill, F.). The Birds of North America, Inc., Philadelphia, PA, 28 pp.

Shi, P. (2001). High-throughput detection of West Nile virus RNA. *J. Clin. Microbiol.* 39, 1264–1271.

Soule, M.E. & Mills, L.S. (1998). No need to isolate genetics. *Science*, 282, 1658–1659.

STATA (2001). STATA version 7.0. STATA Corporation, College Station, TX, USA.

Steele, K.E., Linn, M.J., Schoepp, R.J., Komar, N., Geisbert, T.W., Manduca, R.M. *et al.* (2000). Pathology of fatal West Nile virus

infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet. Pathol.*, 37, 208–224.

United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention (CDC), National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases (2003) *Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control*, 3rd revision. CDC Publication, Fort Collins, CO, USA. Available from: http://www.cdc.gov/ncidod/dvbid/westnile/publications.htm; modified on October 2003.

Weingartl, H.M., Drebot, M.A., Hubalek, Z., Halouzka, J., Andonova, M., Dibernardo, A. *et al.* (2003). Comparison of assays for the detection of West Nile virus antibodies in chicken serum. *Can. J. Vet. Res.*, 67, 128–132.

Westemeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W. *et al.* (1998). Tracking the long-term decline and recovery of an isolated population. *Science*, 282, 1695–1698.

Winterstein, S.R., Pollock, K.H. & Bunck, C.M. (2001). Analysis of survival data from radiotelemetry studies. In: *Radio Tracking and Animal Populations* (eds Millspaugh, J.J. & Marzluff, J.M.) Academic Press, CA, USA, pp. 351–380.

Yaremych, S.A., Warner, R.E., Mankin, P.C., Brawn, J.D., Raim, A. & Novak, R. (2004). West Nile virus and high death rate in American crows. *Emerg. Infect. Dis.*, 10, 709–711.

Editor, Michael Hochberg
Manuscript received 23 April 2004
First decision made 14 May 2004
Manuscript accepted 26 May 2004
Fast track submitted and reviewed