

IDAHO DEPARTMENT OF FISH AND GAME

Virgil Moore, Director

F12AF00801 State Wildlife Research

Final Performance Report



July 1, 2012 to June 30, 2013

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**FEDERAL AID IN WILDLIFE RESTORATION
ANNUAL PROJECT PERFORMANCE REPORT**

State: Idaho

Grant number: F12AF00801

Grant name: State Wildlife Research

Project titles: Statewide Ungulate Ecology
Statewide Bird Ecology

Studies: Statewide Ungulate Ecology

- I** Survival and population modeling of mule deer
- II** Effects of wolf predation on elk and moose populations
- III** Hells Canyon/Lower Salmon River Bighorn Sheep

Studies: Statewide Bird Ecology

- I** Greater-Sage Grouse (*Centrocercus urophasianus*) habitat and population trends in southern Idaho
- II** Pheasant ecology and management
- III** Support for Columbian Sharp-tailed Grouse

Report Period: July 1, 2012 to June 30, 2013

Report due date: September 28, 2013

Location of work: Panhandle, Clearwater, McCall, Southwest, Magic Valley, Southeast, Upper Snake, and Salmon regions (statewide).

Objectives:

Conduct research on mule deer, wolves, elk, moose, bighorn sheep, sage-grouse, pheasants, and sharp-tailed grouse; and their habitats to gather biological information to provide valid, scientifically-based information for wildlife managers to make sound wildlife conservation and management decisions.

If the work in this grant was part of a larger undertaking with other components and funding, present a brief overview of the larger activity and the role of this project.

Studies under Statewide Ungulate Ecology and Statewide Bird Ecology projects are part of a larger wildlife research program. Graduate student projects represent contributing subsets of the main project studies. Overall, the Wildlife Research Program is designed to collect information, analyze results, and disseminate information for wildlife managers and policy makers to assist with conservation and management actions.

Describe how the objectives were met.

STUDIES: STATEWIDE UNGULATE ECOLOGY

STUDY I: Survival and Population Modeling of Mule Deer

Job I: Functional Analysis of NDVI Curves Reveals Overwinter Mule Deer Survival is Driven by Both Spring and Fall Phenology, Not Just Spring

ABSTRACT: Juvenile survival is an important driver of population dynamics of large herbivores. While most studies in seasonal environments have focused on the effects of spring plant phenology on juvenile survival, recent studies demonstrated that fall nutrition plays a crucial role. We tested for both direct and indirect (through body mass) effects of spring and fall phenology on winter survival of 2,315 mule deer fawns across a wide range of environmental conditions in Idaho, USA. We first performed a functional analysis that identified spring and fall as the key-periods for structuring the among-population and among-year variation of primary production (approximated from 1-km AVHRR Normalized Difference Vegetation Index, NDVI) along the growing season. A path analysis showed that early winter precipitation and direct and indirect effects of spring and fall NDVI functional components accounted for 45% of observed variation in overwinter survival. The effect size of fall phenology on body mass was about twice as much as that of spring phenology, while direct effects of phenology on survival were similar between spring and fall. We demonstrate that the effects of plant phenology vary across ecosystems, and that in semi-arid systems, fall may be more important than spring for overwinter survival.

STUDY I, Job II: Estimate the Effect of Habitat Quality of Summer Range on Survival and Recruitment of Fawns

ABSTRACT: Weather conditions and plant composition will determine the nutritional quality of mule deer summer ranges. Nutrition of the adult female and fawns has been shown to influence the growth rate of fawns, a quality strongly related to winter survival. To link nutritional quality to mule deer vital rates, we measured seasonal forage quality and plant composition of adult female summer ranges. Forty-seven phenology plots were initiated in May and June, 2012 in all 3 of the major ecotypes in southern Idaho and revisited 157 times in 2012 and 139 in 2013 during mid to late summer. We collected plant samples at each phenological stage for analysis of crude protein and digestible energy. We will then link actual measured nutritional quality of a vegetation type to remotely sensed measures of NDVI to produce a broad scale estimate of seasonal habitat quality. Plant cameras were deployed at phenology plots as another intermediate step to link RGB levels of the photograph ($n = 48,000$) to the MODIS spectral signature. To understand how summer range selection influence fawn survival, we estimated plant composition of 38 home ranges of adult females with GPS collars distributed across the ecotypes in 2013 (18 in 2012). We averaged 5 transects per home range to adequately characterize the vegetation composition for each deer (85 transects in 2012 and 196 in 2013). Fecal samples were collected in each home range to estimate diet composition

and selection. The summer range specific, nutritional quality estimates will be backcast using the NDVI values to include the years 1998-2013 for which empirical estimates of mule deer fawn survival and fawn ratios are available. Relationships of these vital rates to summer range nutritional quality will be modeled to determine the effect on mule deer population dynamics. To test the importance of different vital rates to population growth, we have developed an integrated population model and are testing it for functionality. Inputs for this model include; age and sex ratios, age specific survival, harvest, and intermittent population estimates. We added modules for estimating survival and visibility bias in aerial surveys in 2013.

STUDY II: Effects of Wolf Predation on Elk and Moose Populations

ABSTRACT: During FY13, we captured 31 elk including 11 cows, and 3 bulls, and 17 calves in the North Fork study area. Thirteen elk collars were recovered from the Lowman site when their GPS successfully dropped on schedule. Two elk were recaptured in the Lowman study area to remove GPS collars and download location data. The final two wolf GPS collars were recovered from the study area. Elk in the North Fork were fitted with GPS radio collars and we obtained various measurements and biological samples. We measured rump fat on captured elk either by palpation or a combination of palpation and ultrasonography. Cow elk serum was evaluated for levels of PSPB to determine pregnancy. We extracted a canine tooth from all elk >3.5 years age and teeth were sectioned to determine age. Elk fecal samples were examined to determine the presence of giant liver fluke ova and all were negative. We captured 13 wolves in the North Fork that were fitted with GPS-Argos radio collars. We conducted monitoring flights on a biweekly basis to determine survival status of elk and wolves. We conducted necropsies on 15 elk and 5 wolves. We downloaded location data from GPS collars obtained from dead animals and collars recovered following release. We currently have 56,532 wolf GPS locations and 560,080 elk GPS locations. We conducted aerial surveys in the North Fork during March to measure the spatial distribution and abundance of elk and alternate ungulate prey. We measured snow depth and sinking depth at 5 sites. We continue to populate an Access database with data from captures, monitoring flights, necropsies, and GPS data from collars.

STUDY III: Hells Canyon/Lower Salmon River Bighorn Sheep

Job I: Hells Canyon Initiative to Restore Rocky Mountain Bighorn Sheep

ABSTRACT: The Hells Canyon Initiative is a state, federal, and private partnership to restore Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) in the Hells Canyon area of Oregon, Idaho, and Washington. During 2012—2013, 138 radio-collared bighorn sheep were monitored regularly in 14 populations. Average annual survival of radio-collared ewes was 0.84 and annual ram survival was 0.78. Pneumonia-caused mortality of lambs was confirmed in 4 populations and suspected in 5 others. The overall metapopulation was estimated at 780 bighorn sheep, an increase from an estimate of 740 sheep in 2011—2012.

Multi-year collaborative projects were continued with the University of Idaho Caine Veterinary Teaching Center (CVTC) and the Department of Veterinary Microbiology and

Pathology at Washington State University to better understand the clinical causes of disease, in particular *Mycoplasma ovipneumoniae*, in bighorn sheep. Three papers were published in peer-reviewed journals.

The goals of the Salmon River bighorn sheep study are to evaluate population and genetic structure of bighorn sheep in the Salmon River drainage in Game Management Units (GMU) 14, 19, 19A, 20, and 20A, and to assess connectivity between these sheep and other Idaho sheep populations. A final report (MS Thesis) is expected by the end of 2013.

STUDY III, Job II: Spatio-Temporal Dynamics of Pneumonia in Bighorn Sheep

ABSTRACT: Bighorn sheep mortality related to pneumonia is a primary factor limiting population recovery across western North America, but management has been constrained by an incomplete understanding of the disease. We analysed patterns of pneumonia-caused mortality over 14 years in 16 interconnected bighorn sheep populations to gain insights into underlying disease processes.

We observed four age-structured classes of annual pneumonia mortality patterns: all-age, lamb-only, secondary all-age and adult-only. Although there was considerable variability within classes, overall they differed in persistence within and impact on populations. Years with pneumonia-induced mortality occurring simultaneously across age classes (i.e. all-age) appeared to be a consequence of pathogen invasion into a naïve population and resulted in immediate population declines. Subsequently, low recruitment due to frequent high mortality outbreaks in lambs, probably due to association with chronically infected ewes, posed a significant obstacle to population recovery. Secondary all-age events occurred in previously exposed populations when outbreaks in lambs were followed by lower rates of pneumonia-induced mortality in adults. Infrequent pneumonia events restricted to adults were usually of short duration with low mortality.

Acute pneumonia-induced mortality in adults was concentrated in fall and early winter around the breeding season when rams are more mobile and the sexes commingle. In contrast, mortality restricted to lambs peaked in summer when ewes and lambs were concentrated in nursery groups. We detected weak synchrony in adult pneumonia between adjacent populations, but found no evidence for landscape-scale extrinsic variables as drivers of disease. We demonstrate that there was a >60% probability of a disease event each year following pneumonia invasion into bighorn sheep populations. Healthy years also occurred periodically, and understanding the factors driving these apparent fade-out events may be the key to managing this disease. Our data and modelling indicate that pneumonia can have greater impacts on bighorn sheep populations than previously reported, and we present hypotheses about processes involved for testing in future investigations and management.

STUDIES: STATEWIDE BIRD ECOLOGY

STUDY I: Greater Sage-grouse (*Centrocercus urophasianus*) Habitat and Population Trends in Southern Idaho

Job I: Factors Influencing the Ecology of Greater Sage-Grouse Inhabiting the Bear Lake Plateau and Valley, Idaho-Utah

ABSTRACT: The purpose of this research is to describe the ecology, seasonal movements, and habitat-use patterns of greater sage-grouse (*Centrocercus urophasianus*) that inhabit the Bear Lake Plateau and Valley relative to existing land-uses. Because the Bear Lake Plateau and Valley is subject to both natural and anthropogenic barriers and fragmentation, defining population vital rates, seasonal movements, and habitat-use relative to land use and jurisdictional boundaries of this population will provide an important foundation for management cooperation among Idaho, Utah, and Wyoming. Preliminary data are provided. Sage-grouse land use research will also define the core use areas of important seasonal and temporal habitats in the Bear Lake Plateau and Valley. The student has completed her field work and is planning on defending her thesis during fall 2013.

STUDY I, Job II: A Method for Estimating Population Sex Ratio for Sage-Grouse Using Non-Invasive Genetic Samples

ABSTRACT: Population sex ratio is an important metric for wildlife management and conservation, but estimates can be difficult to obtain, particularly for sexually monomorphic species or for species that differ in detection probability between the sexes. Noninvasive genetic sampling (NGS) using polymerase chain reaction (PCR) has become a common method for identifying sex from sources such as hair, feathers or feces, and is a potential source for estimating sex ratio. If, however, PCR success is sex-biased, naively using NGS could lead to a biased sex ratio estimator. We measured PCR success rates and error rates for amplifying the W and Z chromosomes from greater sage-grouse (*Centrocercus urophasianus*) fecal samples, examined how success and error rates for sex identification changed in response to fecal sample exposure time, and used simulation models to evaluate precision and bias of three sex assignment criteria for estimating population sex ratio with variable sample sizes and levels of PCR replication. We found PCR success rates were higher for females than males and that choice of sex assignment criteria influenced the bias and precision of corresponding sex ratio estimates. Our simulations demonstrate the importance of considering the interplay between the sex bias of PCR success, number of genotyping replicates, sample size, true population sex ratio and accuracy of assignment rules for designing future studies. Our results suggest that using fecal DNA for estimating the sex ratio of sage-grouse populations has great potential and, with minor adaptations and similar marker evaluations, should be applicable to numerous species.

STUDY I, Job III: Effects of Landscape-Scale Environmental Variation on Greater Sage-Grouse Chick Survival

ABSTRACT: Effective long-term wildlife conservation planning for a species must be guided by information about population vital rates at multiple scales. Greater sage-

grouse (*Centrocercus urophasianus*) populations declined substantially during the twentieth century, largely as a result of habitat loss and fragmentation. In addition to the importance of conserving large tracts of suitable habitat, successful conservation of this species will require detailed information about factors affecting vital rates at both the population and range-wide scales. Research has shown that sage-grouse population growth rates are particularly sensitive to hen and chick survival rates. While considerable information on hen survival exists, there is limited information about chick survival at the population level, and currently there are no published reports of factors affecting chick survival across large spatial and temporal scales. We analyzed sage-grouse chick survival rates from 2 geographically distinct populations across 9 years. The effects of 3 groups of related landscape-scale covariates (climate, drought, and phenology of vegetation greenness) were evaluated. Models with phenological change in greenness (NDVI) performed poorly, possibly due to highly variable production of forbs and grasses being masked by sagebrush canopy. The top drought model resulted in substantial improvement in model fit relative to the base model and indicated that chick survival was negatively associated with winter drought. Our overall top model included effects of chick age, hen age, minimum temperature in May, and precipitation in July. Our results provide important insights into the possible effects of climate variability on sage-grouse chick survival

STUDY I, Job IV: Mapping Sage-Grouse Fence-Collision Risk: Spatially Explicit Models for Targeting Conservation Implementation

ABSTRACT: Recent research suggested greater sage-grouse (*Centrocercus urophasianus*; hereafter, sage-grouse) fence collision may be widespread, and fence-marking methods have been developed for reducing prairie-grouse collision in sagebrush-steppe habitats. However, research also suggested sage-grouse collision was highly variable, and managers implementing mitigation desire targeting tools to prioritize mitigation efforts as a function of risk. We fit collision-risk models using widely available covariates to a sage-grouse fence-collision data set from Idaho, USA, and developed spatially explicit versions of the top model for all known sage-grouse breeding habitats (i.e., within 3 km of leks) in 10 of 11 western states where sage-grouse are found. Our models prioritize breeding habitats for mitigation as a function of terrain ruggedness and distance to nearest lek, and suggest that a relatively small proportion of the total landscape (6–14%) in each state would result in >1 collision over a lekking season. Managers can use resulting models to prioritize fence-marking by focusing efforts on high risk landscapes. Moreover, our models provide a spatially explicit tool to efficiently target conservation investments, and exemplify the way that researchers and managers can work together to turn scientific understanding into effective conservation solutions.

STUDY I, Job V: Greater Sage-Grouse Lek Attendance Rates in Southern Idaho: Visibility Bias for Sage-Grouse Lek Counts

ABSTRACT: Declining breeding populations of greater sage-grouse necessitates careful assessment and accurate estimates of trends at the population level. The primary method for monitoring sage-grouse is by comparing numbers of males observed at groups of traditional lek sites among years. The sightability of male sage-grouse on leks is likely <1 for numerous reasons. The result is termed visibility bias and has generally been

overlooked when considering lek counts. Our objectives were to identify variables that influence sightability of male sage-grouse during lek route surveys and to develop a model to correct for visibility bias. We conducted simultaneous counts of sage-grouse from a truck using standard lek-counting procedures and from blinds positioned ≤ 20 m from the edge of the leks. We built candidate models for predicting visibility bias using logistic regression with variables specific to the lek and time of observation. Sightability in our study varied from $< 50\%$ under certain circumstances to 100% under others. Our analysis revealed strong evidence that sightability of sage-grouse was negatively correlated with lek area, visual obstruction, distance, and time. Alternatively, hen presence increased sightability, as did direct sunlight until 30 min past sunrise. Estimating and correcting for variation in these counts due to visibility bias should improve their power to detect differences among populations or through time, making results more reliable and defensible.

STUDY I, Job VI: Phytochemistry Predicts Habitat Selection by an Avian Herbivore at Multiple Spatial Scales

ABSTRACT: Animal habitat selection is a process that functions at multiple, hierarchically structured spatial scales. Thus multi-scale analyses should be the basis for inferences about factors driving the habitat selection process. Vertebrate herbivores forage selectively on the basis of phytochemistry, but few studies have investigated the influence of selective foraging (i.e., fine-scale habitat selection) on habitat selection at larger scales. We tested the hypothesis that phytochemistry is integral to the habitat selection process for vertebrate herbivores. We predicted that habitats selected at three spatial scales would be characterized by higher nutrient concentrations and lower concentrations of plant secondary metabolites (PSMs) than unused habitats. We used the Greater Sage-Grouse (*Centrocercus urophasianus*), an avian herbivore with a seasonally specialized diet of sagebrush, to test our hypothesis. Sage-Grouse selected a habitat type (black sagebrush, *Artemisia nova*) with lower PSM concentrations than the alternative (Wyoming big sagebrush, *A. tridentata wyomingensis*). Within black sagebrush habitat, sage-grouse selected patches and individual plants within those patches that were higher in nutrient concentrations and lower in PSM concentrations than those not used. Our results provide the first evidence for multi-scale habitat selection by an avian herbivore on the basis of phytochemistry, and they suggest that phytochemistry may be a fundamental driver of habitat selection for vertebrate herbivores.

STUDY I, Job VII: Response of Greater Sage-Grouse to Wind Power Development in Idaho: Final Report

ABSTRACT: The Idaho Department of Fish and Game (IDFG) has been studying this greater sage-grouse (*Centrocercus urophasianus*) population continuously since 2002 for multiple studies and formally initiated research on the impacts of wind power to sage-grouse beginning 1 July, 2011; commencing with the establishment of a funding agreement between IDFG and the Western Association of Fish & Wildlife Agencies (WAFWA) in conjunction with the wind farm developer. The research project involved a Before-After-Control-Impact (BACI) design utilizing both a treatment (Browns Bench) and control (Mary's Creek) area. The treatment area encompassed the proposed China Mountain Wind Farm Project. Funding from the Federal Aid for Wildlife Restoration

was used for supervision of field crews and writing the final report. Seventeen sage-grouse (15 females, 2 males) were captured and fitted with solar-powered satellite platform terminal transmitter (PTT) during 2012. Battery-powered very high frequency (VHF) transmitters were placed on 58 sage-grouse (31 females, 27 males) and monitored for movements, survival, and nest success.

Survival was $83 \pm 16\%$ for females and $73 \pm 33\%$ for males on the proposed treatment area (January–July, 2012). Female survival was $33 \pm 20\%$ and male was $51 \pm 17\%$ on the control area (February–October, 2012). Apparent nest success was 33% on the proposed treatment area and 63% on the control area. Movements were similar to past research projects on both areas and grouse continued to use the proposed wind farm impact zone for seasonal habitat. No further work is being conducted on this project other than retrieval of PTT transmitters as mortalities occur.

STUDY I, Job VIII: Estimating Grass Height at Nest Initiation for Greater Sage-Grouse in Idaho

ABSTRACT: Quality nesting habitat is important for increasing populations of greater sage-grouse (*Centrocercus urophasianus*). Past research has combined grass species and measured after nests hatched. My research focused on individual grass species at nests and random plots. I also calculated grass height at the beginning of incubation based on grass growth within 50 m of 100 nests actively being incubated. Grass structure was measured on 3 drupe height structures (residual, live, flower), number of flower stalks, and horizontal cover (effective height). Percent ground cover was also measured.

Six grass species were the most abundant and occurred at a majority of the nests: Sandberg bluegrass (81% of nests, *Poa secunda*), bluebunch wheatgrass (49%, *Pseudoroegneria spicata*), bottlebrush squirreltail (47%, *Elymus elymoides*), cheatgrass (39%, *Bromus tectorum*), Idaho fescue (17%, *Festuca idahoensis*), and Indian ricegrass (16%, *Achnatherum hymenoides*). Cheatgrass residual height increased 8%, Idaho fescue increased 3% and the rest of the common grasses decreased 6-37% in residual height with Indian ricegrass declining the most. Nest sites had taller residual grass height for two of the four native bunchgrasses (bluebunch wheatgrass and Idaho fescue) than occurred at random. Bluebunch wheatgrass and Sandberg bluegrass had taller live grass height at nests than at random. Bluebunch wheatgrass had taller flower stalks whereas cheatgrass had shorter flower stalks at nests than at random. Nests had significantly more bluebunch wheatgrass ground cover (7.8%) than available at random (5.4%).

Female sage-grouse might be selecting for sites with greater bluebunch wheatgrass because this species provides taller plant structure for concealment of nests. Future work includes developing variables from the dataset that allows for multivariate statistical analysis to determine if nest success is influenced by species specific habitat selection.

STUDY II: Pheasant Ecology and Management

Territorial Male Pheasant Density Response to Habitat Changes

Progress during the performance period was limited to data editing, compilation, and accuracy verification.

STUDY III Support for Columbian Sharp-tailed Grouse Research

Results for this project will be reported in the annual performance report for project W-184-R-1 (Columbia Sharp-tailed Grouse Research) due 31 December, 2013.

Discuss differences between work anticipated in grant proposal and grant agreement, and that actually carried out with Federal Aid grant funds.

The work accomplished during the reporting period corresponds closely with that described in the grant project statement.

List any publications or in-house reports resulting from this work.

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Stevens, B.S., D.E. Naugle, B. Dennis, **J.W. Connelly**, T. Griffiths, and K.P. Reese. 2013. Mapping sage-grouse fence-collision risk: spatially explicit models for targeting conservation implementation. Wildlife Society Bulletin; DOI: 10.1002/wsb.273.

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APPENDIX I
PROGRESS REPORTS FOR STUDIES IN STATEWIDE UNGULATE ECOLOGY
STUDY I: SURVIVAL AND POPULATION MODELING OF MULE DEER

Job I: Functional analysis of NDVI curves reveals overwinter mule deer survival is driven by both spring and fall phenology, not just spring

Submitted for publication in the Philosophical Transactions of the Royal Society B

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Abstract

Juvenile survival is an important driver of population dynamics of large herbivores. While most studies in seasonal environments have focused on the effects of spring plant phenology on juvenile survival, recent studies demonstrated that fall nutrition plays a crucial role. We tested for both direct and indirect (through body mass) effects of spring and fall phenology on winter survival of 2315 mule deer fawns across a wide range of environmental conditions in Idaho, USA. We first performed a functional analysis that identified spring and fall as the key-periods for structuring the among-population and among-year variation of primary production (approximated from 1-km AVHRR Normalized Difference Vegetation Index, NDVI) along the growing season. A path analysis showed that early winter precipitation and direct and indirect effects of spring and fall NDVI functional components accounted for 45% of observed variation in overwinter survival. The effect size of fall phenology on body mass was about twice as much as that of spring phenology, while direct effects of phenology on survival were similar between spring and fall. We demonstrate that the effects of plant phenology vary across ecosystems, and that in semi-arid systems, fall may be more important than spring for overwinter survival.

Key words: Demography, NDVI, Phenology curve, Population dynamics, Ungulate, Winter severity

1. INTRODUCTION

Like most other large herbivores of temperate and northern areas, mule deer (*Odocoileus hemionus*) population growth is more sensitive to a given change in adult female survival than to the same change in other demographic parameters, although adult female survival tends not to vary in mule deer [1, 2]; [3] for a general discussion. In contrast, juvenile survival shows the widest temporal variation, often in response to variation in weather [4-6] and population density [7]. This large variation in juvenile survival, especially overwinter survival, often drives population growth of mule deer [1, 2, 8]. Fawns accumulate less fat than adults during the summer, which increases their mortality because variation in late summer nutrition interacts with overwinter severity [1, 9]. While previous studies have shown that spring NDVI correlates with early juvenile survival, summer survival is only one component of recruitment, and is not necessarily more important than overwinter survival to shape overall juvenile survival. Yet, to date, the effect of changes in fall plant phenology on overwinter juvenile survival remains unexplored.

Focused by the pioneering experimental work on elk (*Cervus elaphus*) [10], there is a growing recognition that in temperate areas, late summer and fall nutrition are an important driver of overwinter survival and demography of large herbivores [10, 11]. Lactation during summer is a nutritionally demanding part of the annual nutritional cycle [12, 13]. Summer nutrition first affects adult female body condition [11], which predicts pregnancy rates [10, 11, 14], overwinter adult survival rates [11, 15], litter size [16], as well as birth mass and early juvenile survival [8, 16, 17]. Nutrition during winter (energy) minimizes body fat loss [8], but rarely changes the importance of late summer and fall nutrition for survival of both juveniles and adults [10]. Winter severity interacts with body condition to shape winter survival of ungulates [11, 18], and can, in severe winters, overwhelm the effect of summer/fall nutrition in driving overwinter survival of juveniles.

There has been a recent explosion of the integration of remote sensing data into wildlife conservation and management [19-21]. With satellites like AVHRR, MODIS, SPOT [22, 23], and growing tool sets for ecologists [24], derived metrics are being commonly used to analyze the ecological processes driving wildlife distribution and abundance [21]. Indices such as the Normalized Difference Vegetation Index (NDVI) and the Enhanced Vegetation Index (EVI) strongly correlate with vegetation productivity, track growing season dynamics [25, 26] and differences between landcover types at moderate resolutions over broad spatio-temporal scales [22]. For large herbivores NDVI has become the gold standard for indexing habitat quality across large spatio-temporal scales [21, 27, 28]. This is because large herbivores select for intermediate forage biomass to maximize forage quality [29]. Phenological parameters extracted from NDVI correlate with maximum forage quality for a variety of ungulates [27, 30, 31]. As a result of the link between NDVI metrics and forage quality, NDVI can be predictive of ungulate nutrition [27], home range size [32], migration [28], and movements [31, 33]. An increasing number of studies have also linked NDVI to body mass and demographic parameters of vertebrates. Most of these studies (reviewed in Table 1) only focused on just spring phenology, although recent field studies on ungulates emphasized the critical importance of late summer and fall nutritional ecology. Moreover, all but one [34] were based on pre-defined metrics assumed to provide a reliable description of the NDVI curve.

Thus, ecologists have increasingly used a number of standardized growing season parameters describing the onset, peak, and sensation of plant growth. Unfortunately, these useful parameters are often highly correlated. In Wyoming for instance, the start of the growing season

was delayed and the rate of green-up decreased following winters with high snow cover [35]. Thus, one main barrier to understanding the complex influence of growing season dynamics on ungulate survival is how to disentangle correlated plant phenology metrics. Another underappreciated barrier is the challenge of harnessing the time series nature of NDVI data, which requires appropriate specific statistical tools, but that few previous studies have addressed. This explains why no previous study has attempted to describe the entire NDVI function across the entire growing season. To fill this important gap, the joint use of functional analysis [36] to characterize seasonal variation in NDVI curves and path analyses [37] to assess both direct and indirect effects of plant phenology offers a powerful way to address such entangled relationships.

Our first goal was to identify the key-periods for structuring the among-population and among-year variation of primary production, measured remotely using NDVI curves along the growing season. Second, with the main periods of NDVI variability identified, we assessed both direct and indirect (through fawn body mass) effects of these key-periods on overwinter mule deer fawn survival. We took advantage of a uniquely long-term (1998 – 2011) and large spatial scale approach to disentangle plant phenology effects on mule deer survival, encompassing 13 different populations spread over Idaho while most previous studies have focused only within 1 or 2 populations. These populations represent diverse ranges of elevation, habitat quality, and climatological influences. We focus here on overwinter fawn survival because previous studies [1, 2] have demonstrated that this parameter is of paramount importance driving overall population growth rate of mule deer. However, the influences of plant phenology during the growing season and of winter severity on winter survival are not independent because they both involve a strong indirect effect of body mass. Mysterud *et al.* [38] used a path analysis to separate independent effects of summer versus winter effects on body mass and survival. We propose a novel methodological framework in which we analyze direct remote sensing NDVI measurements using functional principal component analysis in an effort to discriminate regions with differing fall and spring phenology. We then use Hierarchical Bayesian path analysis approach to identify drivers of overwinter mule deer survival. We predicted, based on previous studies, that plant phenology should drive body mass of mule deer at 6 months of age, and that body mass and winter severity should be the primary drivers of overwinter survival. We expected direct effects of plant phenology on winter survival to be weaker. We also expected early winter severity would affect overwinter fawn survival more than late winter severity based on previous studies [39].

2. Materials and methods

(a) Study Areas

The study area spanned $\sim 160,000\text{km}^2$, representing nearly the entire range of climatic conditions and primary productivity of mule deer in Idaho. We focused on 13 populations with winter ranges corresponding to 13 Idaho game management units (GMUs); hereafter we use GMU synonymous with population (Fig. 2). All deer populations were migratory with an average winter range size (see (b) mule deer monitoring below) for each GMU was 430km^2 and average summer range size of 3360km^2 . There are three main habitat types (called ecotypes hereafter) within the study area based on the dominant overstory canopy species on summer range, including; coniferous forests, shrub-steppe, and aspen woodlands. The populations were distributed among the ecotypes with 5 populations mostly in conifer in the western or north-central portion of the state (GMUs 32, 33, 36B, 39, 60A), 2 in shrub-steppe ecotype in the central to eastern area (GMUs 54, 58), and 6 in aspen primarily in the east and south (GMUs 56, 67, 69,

72, 73A, 76). Elevation and topographic gradients within GMUs affect snow depths and temperature in winter, and precipitation and growing season length in the summer. The general gradient of elevation increased from the southwest to the North and East. Conifer GMUs ranged in elevation from 1001 – 1928m, but most were <1450m. Winter precipitation (winter severity) varied widely (from 10 to 371mm) in coniferous GMUs. Coniferous ecotype summer ranges are dominated by conifer species interspersed with cool season grasslands, sagebrush, and understory of forest shrubs. Shrub-steppe GMUs ranged from 1545 to 2105 m, with winter precipitation from 24 to 105 mm. Summer range within shrub steppe ecotypes was dominated by mesic shrubs (e.g., bitterbrush (*Purshia tridentata*) sagebrush spp. *Artemisia*, rabbitbrush *Chrysothamnus* spp., etc). Aspen ecotype GMUs were located in the east and south with winter use areas ranging from 1582 to 2011m, with 5 of the 6 GMUs above 1700m with early winter precipitation ranging from 25 to 146mm. In summer, productive mesic Aspen (*Populus tremuloides*) woodlands were interspersed with mesic shrubs.

(b) Mule deer monitoring

We radiocollared mule deer fawns at 6 months of age in the 13 GMUs (Fig. 1), resulting in 2315 mule deer fawns from 1998 – 2011. We captured fawns primarily using helicopters to move deer into drive nets [40], but occasionally by helicopter netgun [41] or clover traps [42]. Mule deer capture and handling methods were approved by IDFG (Animal Care and Use Committee, IDFG Wildlife Health Laboratory) and University of Montana IACUC (protocol #02-11MHCFC-031811). Fawns were physically restrained and blindfolded during processing with an average handling time of < 6 minutes. Collars weighed 320 - 400 grams ($\leq 2\%$ of deer mass), were equipped with mortality sensors and fastened with temporary attachment plates or surgical tubing, allowing the collars to fall off the animals after approximately 8-10 months. We monitored between 20 and 34 mule deer fawns in each study area for a total of 185 to 253 annually from 1998 – 2011. We measured fawn mass to the nearest 0.4 kilogram with a calibrated spring scale.

We monitored fawns with telemetry for mortality from the ground every 2 days between capture and 15 May through 2006, and then once at the 1st of each month during 2007-2011. Missing fawns were located aerially when not found during ground monitoring. When a mortality signal was detected, we determined cause of death using a standard protocol [43] and included all sources of mortality in survival analyses. In addition, we kept a minimal annual sample of ~ 600 adult females with radiocollars, using the same capture techniques as fawns. We used aerial locations from these deer to estimate herd unit home ranges.

(c) Defining Mule Deer Population Ranges

We used the mule deer winter range for each GMU as the main spatial unit of analysis, and we extracted NDVI data and winter weather for each year from each population. Relocation points for all individuals and years in a single study site were combined to estimate a 95% adaptive kernel home range for both summer and winter [44] for mule deer captured within a population. Migratory periods, 1 April to 1 June and 1 October to 15 November, were excluded from the home range estimates, animal locations between 1 June and 30 September were used for summer, 1 December to 31 March for winter. Climate and habitat information was then summarized by the aggregate home range of radio-collared deer for winter and summer within each population.

(d) Functional Analysis of NDVI curves

We measured growing season phenology for each population-year using the NDVI [45]. We used the 1-km resolution AVHRR conterminous United States and Alaska NDVI data obtained from the NOAA-14,-16, and -17 AVHRR satellites, a 1km, 7-day composite dataset compiled by [46] and maintained by the USGS (<http://phenology.cr.usgs.gov/index.php>) from 1997 – 2010 (e.g., NDVI from the growing season preceding winter). Because our study period extended beyond the period of MODIS availability, we opted to use the continuously available AVHRR composite product, though this AVHRR dataset and MODIS show strong correspondence [47]. This dataset was corrected for radiometric sensor anomalies, cloud cover contamination and geometric registration accuracy (Eldenshink [46]). Phenological changes in NDVI only directly represent ungulate forage dynamics in non-forested vegetation types, thus we extracted NDVI values from (non-burned) grass and shrub community types characterized by the SAGEMAP (2005 USGS, Forest and Rangeland Ecosystem Science Center, Snake River Field Station, Boise, ID) and North West Regional GAP analysis models of vegetation cover within each of the mule deer ranges. To encompass the entire growing season for each population-year, but excluding winter anomalies caused by varying snow condition, we restricted NDVI data to 15 March to 15 November. This time period provided a standardized measure of growing season while capturing the variability both within and between populations for comparing curves.

We first assessed among population-year variation in NDVI curves to test direct and indirect (i.e., through body mass) effects of changes in plant phenology on overwinter survival of fawns. In most previous studies (see table 1 for a review), ecologists have either used a-priori summary statistics of NDVI, or extracted a-priori defined phenology variables such as peak of NDVI, integrated NDVI, or timing of maximum rate of change in spring NDVI [20]. Unfortunately, while elegant, this approach has led to the use of only a few variables to define the growing season in any ecosystem. As we do not expect patterns of variation in plant phenology along the growing season to be the same everywhere, we proposed a new approach to identify the key-periods along the NDVI curve. Instead of defining these periods a priori, our approach is based on a multivariate functional analysis of variation in observed NDVI curves. We used a functional principal component analysis (FPCA), a particular type of functional data analysis (FDA) to analyze among-population and among-year variation in NDVI curves. FDA is specifically designed to describe, summarize and model information in multivariate time series [36]. FPCA techniques are relatively recent [36] and surprisingly rarely used in ecology (but see [48] for a recent application) even if they offer a very powerful way to analyze temporal ecological data such as NDVI time series. FPCA was applied to NDVI curves to identify the main patterns of vegetation changes and how these temporal trends vary across space. While a-priori defined metrics estimated from NDVI data has sometimes been treated by a standard PCA to identify the main patterns vegetation [49], standard PCA is not optimal for time series data. Indeed, weeks would be considered as independent (and thus interchangeable) vectors of values whereas FPCA takes explicitly into account the chronology of weeks. Hence, in FPCA, the statistical units (in rows) is the whole NDVI curve rather than a vector of NDVI values irrespective of time like in standard PCA. The main advantage of FPCA is that similarities among populations are thus measured using the full NDVI curves while standard PCA would only consider sets of date-specific NDVI values. This ensures that the patterns identified by FPCA are constrained to be temporal trends within the growing period (i.e., portions of the curve) and not due to few independent NDVI values. As in standard PCA, FPCA produces eigenvalues (that measures the amount of variation explained by each dimension) and principal

component scores for sampling units (that summarize the similarities among NDVI curves). However, eigenvectors are replaced by eigenfunctions (harmonics) that show the major functional variations associated to each dimension.

To facilitate the application of FPCA by ecologists we provided in electronic supplementary materials the data and the full R code (based on the `fda` package) to reproduce the analysis performed in the paper. As these methods are poorly known in ecology, we also provided an expanded description of the mathematical theory but the reader could consult the original books by [36, 50] for additional information.

Sampling units (population-years) were partitioned using the k-means algorithm applied on the first two principal component scores. We computed the Calinski & Harabasz criterion for partitions between 2 and 7 groups and select the optimal number of clusters that maximizes the criterion. We also computed the amount of variation in the first two principal component scores (NDVI curves) that were explained by space (i.e., population) and time (year). This allowed us to understand which source of variation contributed most to differences in growing season dynamics. We then used principal component scores in subsequent analyses as explanatory variables of mule deer fawn mass and survival.

(e) PRISM Weather Data

We characterized winter (1 November to 31 March) weather conditions using 4km gridded PRISM observations of minimum monthly temperature and total monthly precipitation from 1995 – 2011 [51](available from <http://www.prism.oregonstate.edu>). Temperature and precipitation data were averaged across the winter range for each population, and then summed (averaged) across months for precipitation (temperature) to produce climate covariates that represented measures of winter severity, respectively. We produced variables for early winter (November – December) and late winter (January – March) for both precipitation and temperature. These variables were highly correlated ($r > 0.4$), thus we selected the variable with the highest first order correlation to our response variable, overwinter survival of fawn, as our winter severity index.

(f) Environmental Effects on Body Mass and Overwinter Survival of Fawns

We estimated population- and year-specific estimates of overwinter fawn survival (from 16 December to 1 June) using staggered Kaplan-Meier non-parametric survival models. We then employed path analysis [37] to test the population-level effects of body mass and winter weather, and to tease apart the direct from the indirect effects (through fawn body mass, see Fig. 3) of key periods of NDVI on overwinter survival. For the path analysis, we transformed our response variable with an arcsin-square root function because survival is obviously bounded between 0 and 1 [52]. We used mass of female fawns in December to measure the cohort quality of the birth year [53] to account for sexual size dimorphism [2]. A first, indirect, mechanistic link between environmental conditions early in life and overwinter survival could be that variation in NDVI curves affects the body development of fawns, which in turn, drives overwinter survival. An alternative could be that variation in NDVI curves is directly related to overwinter survival as a result of the availability and quality of winter forage. Because winter precipitation was recorded in November-December at the same time as the weighing of fawns, we could not test for an indirect effect of winter precipitation through body mass on overwinter survival. Our model included a population effect entered as a random factor on the intercept to account for the repeated measurements of overwinter survival in different years within a population.

We used a Bayesian framework to fit the path analyses to our data [54]. We used non-informative normal (mean of 0 and a standard deviation of 100) and uniform (range between 0 and 100) priors for the regression coefficients and variance parameters respectively. Using JAGS [55], we generated 50 000 samples from Monte-Carlo Markov Chains (MCMC) to build up the posterior distributions of estimated parameters after discarding the first 5 000 iterations as a 'burn in'. We checked convergence graphically and based on Gelman's statistics [54]. Estimated parameters were given by computing the mean of the posterior distribution, and the 2.5th and 97.5th percentiles of the distribution provided its 95% credibility interval. We considered a variable as statistically significant if the credibility interval of its posterior distribution excluded 0. We assessed the fit of the model by computing the squared correlation coefficient between observed and predicted values [56]. Finally, to compare the relative effect sizes of the explanatory variables on overwinter survival we replicated the analyses using standardized coefficients.

3. Results

(a) Functional Analysis of NDVI Curves

Functional PCA analysis of NDVI data led to the identification of two independent eigenfunctions (hereafter FPCA components), which reflected contrasting patterns of plant phenology in spring and fall. Both FPCA components corresponded to continuums of increasing NDVI intensity, in early and late growing seasons, and were used as explanatory variables of overwinter survival of mule deer fawns.

The first FPCA component described the late season phenology, after peak value and accounted for 51.4 % of the total variation in NDVI curves. The second FPCA component represented the early season phenology and accounted for approximately half as much variation as the first FPCA component (27.6 %; Fig. 1). FPCA components can be interpreted as the amount of deviation from the overall average NDVI curve in terms of overall primary productivity at different times within the growing season. For example, high FPCA component 1 scores mean both high primary productivity in open habitats in fall, but also a longer fall growing season compared to lower FPCA component 1 scores (Fig. 1a, c). Similarly, positive values of FPCA component 2 reflect both higher spring primary productivity and early onset of plant growth (e.g., Fig. 1b, c; Type 4 dark green).

Combining both continuums in a factorial plane allowed us to distinguish five NDVI types of curves in reference to the overall mean trend (Fig. 1c). For example, NDVI in fall was close to the average for the NDVI curve type 3 (light green, Fig. 1c) but NDVI in spring was the lowest of all curve types in Fig. 1c. Conversely, NDVI curve type 2 (dark blue, Fig. 1c) has NDVI values above average in both spring and fall. The NDVI curve type 1 (light blue, Fig. 1c) has the highest NDVI in fall, while NDVI curve type 5 (red, Fig. 1c) had lowest fall productivity. Generally, a given population fell into one NDVI curve type, with some extreme values belonging to a different type (Fig. 2, see also supplemental figure S1). Decomposition of the among-population and among-year variance in NDVI curves in fact shows that most (70%) of the observed variation in NDVI curves was accounted for by population (i.e., spatial variation), and much less (25%) by annual variation within a population, with a high degree of synchrony between populations within a year (only 5% of the variation in NDVI curves is unexplained). This suggests that the 5 NDVI types we identified (Fig. 1) strongly reflect the distribution of ecotypes and vegetation characteristics among populations (Fig. 2).

(b) Environmental Effects on Body Mass and Overwinter Survival of Fawns

The average body mass of female fawns in December was 34.0 kg (SE = 2.55). In agreement with our hypothesis, body mass of 6-month-old fawns was positively related to NDVI in both spring and fall (Fig. 3 & 4). From the estimated standardized regression coefficients, the effect of NDVI in fall (FPCA component 1) on autumn body mass of fawns ($\beta = 0.888$, SE = 0.201) was twice the effect of NDVI in spring (FPCA component 2; $\beta = 0.444$, SE = 0.0195). The fall was thus of more importance to the body development of mule deer fawns at the onset of winter than spring (Fig. 3 & 4).

The annual overwinter survival of mule deer fawns averaged 0.55 (SE = 0.24, range = 0 to 0.94) across populations. Our best model accounted for 44.5% of the observed variation in overwinter survival, including the additive effects of autumn body mass of female fawns, early winter precipitation, and of spring and fall NDVI. As expected when mean body mass reflects the average demographic performance of a given cohort, the annual overwinter survival of fawns was associated positively with the mean cohort body mass in late autumn (Fig. 3 & 5A). Total precipitation during early winter from November to December (ranging from 11 to 372 mm) was associated with decreased fawn survival (Fig. 3 & 5B). Once the effect of body mass and winter precipitations were accounted for, spring and fall had both counterintuitive negative impacts on the overwinter survival of fawns (Fig. 3, 5C & 5D) so that the greater the NDVI during the plant growth season, the lower overwinter survival was. Winter precipitation has the greatest effect size on overwinter survival of fawns (standardized $\beta = -0.238$, SD = 0.040), followed by spring (standardized $\beta = -0.123$, SD = 0.045) and fall (standardized $\beta = -0.096$, SD = 0.052), while fawn body mass in fall has the weakest relative effect size (standardized $\beta = 0.071$, SD = 0.030). The observed relationships between environmental conditions and overwinter survival of fawns differed slightly among populations but differences were not statistically significant (electronic supplemental materials).

4. Discussion

Our results linked among-populations and among-year variation observed in plant phenology to that observed in body mass and survival of juvenile mule deer during winter. We used a new statistical approach to identify the key-periods of plant phenology from NDVI curves encompassing the entire growing season. Most previous studies have reported effects of NDVI on body mass and demographic parameters in several species of mammals and birds (see table 1 for a review). However, all these studies but [34]'s one were based on a-priori defined metrics mostly focusing on spring, explaining why spring is generally considered to be the critical period for early life performance in populations of large herbivores (see Table 1). By defining the critical periods a-posteriori, we found that mule deer fawns survived better in populations with higher NDVI during fall, and thus longer fall growing seasons, in open canopied habitats. The effect size of fall NDVI on winter fawn survival was twice as strong in predicting 6-month old body mass as the effect size of spring NDVI. Body mass was, as expected, positively related to overwinter survival, but winter severity during early winter decreased survival with an effect size almost 3 times as strong as body mass. Previous studies on large herbivores reported an effect of the preceding winter conditions when the juvenile was in utero [34, 38, 49, 57], or an effect of spring conditions [49] on body mass. As expected, winter severity had the strongest effect on overwinter survival, similar to many previous studies of winter ungulate survival [2, 58, 59]. Our results highlight the importance of primary production during fall within a semi-arid ecosystem compared to previous studies emphasizing spring. The patterns of variation in NDVI

curves translated to spatial variation in plant growth during fall, and hence mule deer body mass and survival. First, we found almost twice as much variation in the NDVI curves occurred in the fall (FPCA component 1, Figure 1a) compared to spring (FPCA component 2, Figure 1a). Thus, plant phenology during the fall was more variable than spring in our semi-arid system. Second, we found almost three times the variation in NDVI curves was explained by spatial variation among populations in a given year compared to among-year variation. The high proportion of the variance explained among populations indicates that variation among NDVI curves within a population was consistent year-to-year and also synchronous between units within a year. These patterns of stronger variation during fall (vs. spring) and among populations (vs. among years) contributed to fall NDVI having double the effect size on body mass, and hence survival. Thus, the most variable period of the growing season (e.g. fall) had the strongest effect size on mass and survival. These results mirror results from studies of just the spatial variance in survival (Lukacs *et al.* 2009), and suggest that plant phenology may also synchronize population dynamics. With the recent focus on fall nutrition of elk [10], however, many ungulate managers in North America are focusing increasingly on fall nutrition. Our results emphasize that, at least for large herbivores, focusing a priori on just one season without a specific analysis of the spatiotemporal variation in the entire curve of plant phenology could be misleading.

Forage availability for large herbivores varies by habitat and with precipitation and temperature during the growing season [14, 60]. Our results of increased body mass at the onset of winter and hence, overwinter survival with high NDVI in late summer-fall, the period of lowest rainfall in the arid intermountain west, highlights the importance of forage available to fawns during and post weaning. Increased rainfall in summer, reflected in increased NDVI in fall, will promote growth of forbs [60] a highly selected forage for mule deer [60, 61] can promote new growth in fall germinating annual graminoids (e.g., Cheatgrass *Bromus tectorum*) and delay senescence of herbaceous forage and shrubs, all of which prolongs the access of ungulates to higher quality forage [31]. Increased summer-fall nutrition improved calf and adult female survival, fecundity rates, and age of first reproduction in captive elk [10]. Rainfall during the growing season also increases quality and quantity of winter forage [60], which increases survival of fawns and adult female mule deer [8]. Tollefson *et al.* [16] showed that summer forage has the greatest impact on mule deer juvenile survival and overall population growth rate. In Kruger National Park, while most species abundances were more strongly affected by wet season precipitation, several species were more strongly affected by dry season precipitation [62]. Therefore, especially in arid or semi-arid systems, we expect that future studies will identify strong signatures of fall NDVI and climate on demographic parameters of large herbivore populations.

One obvious difference between our ecosystem and previous studies of NDVI and large herbivores is that NDVI curves were not a classic bell shape. Instead, plants in open-habitats have a very left-skewed growth curve, with a rapid green up in spring, but then a long right tail in the NDVI distribution, and, occasionally, secondary growth peaks in late summer and fall (e.g., Fig. 1c). Most other studies that examined NDVI curves found more symmetric shapes, with a rapid plant green up and senescence [49, 63]. However, [64] found the classic bell-shaped NDVI curve for Norwegian and Scottish Red deer, but a similarly earlier and flatter NDVI curve in southern Spain. This may also be because previous studies did not extract NDVI only in open habitats in combination with landcover data. Open habitats are more reflective of ungulate forage quality [65]. If there are differences between phenology of trees versus herbaceous landcover, this may explain our left-skewed growing season distribution. Nonetheless, the variability among

studies in the shape of the NDVI curves emphasizes the importance of identifying key periods of the growing season a-posteriori.

Functional analysis provides a powerful approach to identify the key-periods of the growing season and to assess their differential effects on life history traits. Our functional analysis applied to year- and population-specific NDVI curves allowed us to identify two distinct components of variation that corresponded closely to contrasting spring and fall phenology. Of course, many remote sensing studies have used NDVI for decades to examine spring and fall phenology differences [22]. Yet functional analysis allowed us to identify key periods a-posteriori and to summarize NDVI curves into only 2 independent components instead of 5-12 a-priori defined metrics that are strongly correlated (see Table 1). Functional analysis thus appears to provide a novel approach, applicable to studies of the ecological effects of NDVI (see supplemental materials for R code and NDVI data used in this paper for an example).

One puzzling result from our study was the negative direct effects of both spring and fall NDVI on overwinter survival of mule deer fawns. Spring NDVI had a slightly greater direct effect size on overwinter survival than fall NDVI, in contrast to the stronger effect size of fall NDVI on body mass, and of body mass on overwinter fawn survival. There could be several competing explanations for this puzzling result. First, despite the inherent power of path analysis at disentangling complex relationships (Shipley 2004), there could still remain some confounding effects of body mass. There could also be negative covariance between winter severity, which, because spring NDVI is correlated to winter severity of the preceding winter [35], could lead to negative correlation between spring NDVI and subsequent winter severity. Another potentially important mechanism could be the process of viability selection operating on mule deer cohorts [66]. Counter-intuitively, if good spring growing conditions enhance summer survival, a large proportion of the cohort will survive until the onset of the winter, including frailty [67] individuals with short life expectancy (often considered to be low quality individuals, [68]. On the other hand, only a small proportion of fawns will survive to the onset of winter in cohorts born under harsh spring conditions. As individual early mortality in populations of large herbivores is tightly linked with maternal condition and home range quality ([5]), fawns surviving to the winter will be mostly high quality fawns enjoying rich maternal home ranges. Those fawns would thus be expected to be robust enough to survive winter. Bishop et al. [8] suggested this exact viability selection process for mule deer fawns in Colorado, supporting our interpretation of this counterintuitive spring NDVI effect. Viability selection could also be compounded through the interaction between winter severity and predation, important for mule deer in our study area given the preponderance of predator-caused mortality in winter [2]. There might also be negative covariance between neonate and overwinter survival [8], driven as we suggest here by different spring and fall phenology patterns.

In conclusion, in large parts of world that are semi-arid or deserts, our results strongly show that it may not be just spring phenology that matters to ungulate population dynamics. Our new approach using functional analysis of the entire NDVI curve provides a powerful method to identify first key periods within the growing season and then disentangle their respective role when combined with hierarchical path analysis. Our approach thus allowed us to determine the most likely pathways that plant growth influenced mule deer overwinter survival of fawns. We showed that the among-year and among-population variation in plant phenology involves more than simply spring phenology alone, and we suspect this will be true for many other ecosystems.

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Table 1. A brief literature survey of the studies that investigated relationships between NDVI metrics and life history traits linked to performance and population abundance. The literature survey was performed using ISI web of knowledge using the key-words “NDVI and survival”, “NDVI and body mass”, “NDVI and body weight”, “NDVI and reproductive success”, “NDVI and recruitment”, “NDVI and population growth”, and “NDVI and population density”. Only studies performed on vertebrate species were retained. For each case study, the table displays the focal trait(s), the focal species, the NDVI metric(s) used, the outcome (“+”: positive association between NDVI and performance, “-”: negative association between NDVI and performance”, “0”: no statistically significant association between NDVI and performance”), the reference, and the location of the study.

Trait	Species	NDVI metrics	Outcome	Location	Reference
Protein mass Body mass Carcass mass Body fat	Caribou <i>Rangifer tarandus</i>	Average NDVI in June	Protein mass: + Other traits: 0	Québec-Labrador (Canada)	[69]
Birth mass Juvenile autumn mass	Caribou <i>Rangifer tarandus</i>	Average NDVI in June	+	Québec-Labrador (Canada)	[70]
Population density Juvenile body mass	Semi-domesticated reindeer <i>Rangifer tarandus</i>	Summed NDVI over the breeding season	Juvenile mass: 0 Population density: + (in populations with poor winter ranges only)	Norway (across populations)	[71]
Population size	Lesser grey shrike <i>Lanius minor</i>	NDVI in May-June (breeding areas) NDVI in January- March (wintering areas)	+	France Spain (breeding areas) Kalahari (wintering areas)	[72]
Reproductive performance (lamb/ewe in December)	Sheep <i>Ovis aries</i>	NDVI in March-May NDVI in May	NDVI in May: + NDVI in March-May: 0	North Western Patagonia	[73]
Survival	African elephant <i>Loxodonta africana</i>	Seasonal maximum NDVI	Juvenile survival: + Adult survival: 0	Kenya	[74]

Table 1 Continued

Trait	Species	NDVI metrics	Outcome	Location	Reference
Parasite loading	Red-legged partridge <i>Alectoris rufa</i>	Yearly mean NDVI	+	Spain	[75]
Body mass	Red deer <i>Cervus elaphus</i>	Monthly NDVI	Spring NDVI: + (Spain only) Other metrics/populations: 0	Europe (across population)	[64]
Wing length Tail length Clutch size Body mass (Males and females)	Barn swallow <i>Hirundo rustica</i>	NDVI in December- February (wintering areas)	Male wing length, male and female tail length, clutch size: + Other traits: 0	Italy (breeding area) Africa (wintering areas)	[76]
Juvenile and adult Survival	White stork <i>Ciconia ciconia</i>	NDVI in October- November (Sahel) NDVI in December- February (Eastern southern Africa)	+	Eastern Germany Poland (breeding areas)	[77]
Adult survival	Barn swallow <i>Hirundo rustica</i>	NDVI in September- November NDVI in December- February NDVI in March-May (wintering areas in Africa)	+	Denmark	[78]
Conception rates	African elephant <i>Loxodonta africana</i>	Seasonal NDVI (wet vs. dry seasons)	+	Kenya	{Wittenmyer et al. 2007 ; Rasmussen et al. 2006}

Table 1 Continued

Trait	Species	NDVI metrics	Outcome	Location	Reference
Juvenile and adult survival	Egyptian vulture <i>Neophora percnopterus</i>	Yearly NDVI (wintering areas) NDVI in September-June (breeding areas)	+	Spain	[79]
Survival Reproductive success	Red-backed shrike <i>Lanius collurio</i>	NDVI in September-October (Sahel) NDVI in December-March (South Africa) NDVI in April (Germany)	Survival: + (NDVI in December to March) Reproductive success: + (NDVI in September-October)	Germany	[80]
Juvenile survival	Greater sage grouse <i>Centrocercus urophasianus</i>	NDVI in May-August NDVI and Max NDVI in May, June, July, and August	+ (trends only) Strong co-variation among NDVI metrics	Idaho Utah (USA)	[81]
Body mass	Red deer <i>Cervus elaphus</i>	NDVI in the 1 st of May	+	Norway	[82]
Juvenile body mass	Roe deer <i>Capreolus capreolus</i>	Summed NDVI in April-May Summed NDVI in August-October	+ (Chizé population) 0 (Trois Fontaines population)	France	[83]
Kidney mass	Hystriognath rodents	Yearly NDVI (calculated from monthly NDVI)	-	South America (across species)	[84]
Body mass	Moose <i>Alces alces</i>	7 NDVI metrics (PCA)	+	Norway	[49]
Body mass	Wild boar <i>Sus scrofa</i> Roe deer <i>Capreolus capreolus</i>	Summed NDVI over the growing season	Roe deer: 0 Wild boar: 0	Poland	[85]

Table 1 Continued

Trait	Species	NDVI metrics	Outcome	Location	Reference
Body condition	Raccoon dog <i>Nyctereutes procyonoides</i>	4 NDVI metrics (onset of spring, peak NDVI, Summed NDVI over the growing season, and rate of NDVI increase in spring)	Onset of spring: - Peak NDVI and Summed NDVI: + Rate of NDVI increase: 0	Finland	[86]
Juvenile body mass Reproductive success	Reindeer <i>Rangifer tarandus</i>	EVI (Enhanced Vegetation Index) Modelled using a double logistic function. Use of the parameters S (onset of spring), mS (rate of EVI increase), and mEVI (plant productivity)	S and mEVI on both mass and reproductive success: +	Norway	[34]
Juvenile body mass Pregnancy rate	Elk <i>Cervus elaphus</i>	NDVI correlated with bi-weekly forage biomass and quality over the previous growing season	Exposure to higher predicted forage quality : + juvenile body mass + female pregnancy	Canada	[31]
Juvenile mass	Sheep <i>Ovis aries</i> (2 breeds)	NDVI in late May Summed NDVI in June-August	NDVI in late May: + Summed NDVI in June-August: 0 or – depending on the breed	Norway	{Nielsen et al. 2012 ; Nielsen et al. 2013}

Table 1 Continued

Trait	Species	NDVI metrics	Outcome	Location	Reference
Population size	Common House-Martin <i>Delichon urbicum</i> Common swift <i>Apus apus</i>	NDVI in December-February (wintering areas in Africa)	+	Italy	[87]
Juvenile body mass	Chamois <i>Rupicapra rupicapra</i>	5 NDVI metrics (NDVI slope in spring, NDVI maximum slope in spring, maximum NDVI, date of NDVI peak, Summed NDVI in March)	+ (Summed NDVI in March the best predictor)	France	[88]
Juvenile growth Juvenile survival	Mountain Goat <i>Oreamnos americana</i> Bighorn sheep <i>Ovis canadensis</i> Alpine ibex <i>Capra ibex</i>	Summed NDVI in May Summed NDVI over the growing season Rate of NDVI change	Rate of NDVI change: - Other metrics: 0	Canada Italy	[89]
Population abundance	American redstarts <i>Setophaga ruticilla</i>	NDVI in December-March (wintering areas)	+	North America (breeding areas) Caribbean – Cuba (wintering areas)	[90]
Reproductive success Survival	White-tailed deer <i>Odocoileus virginianus</i>	Summed NDVI in May-August Rate of NDVI change between May and June Maximum change between May and	Summed NDVI in May-August on reproductive success: + Rate of NDVI change	Anticosti Québec (Canada)	[91]

Table 1 Continued

		June	and Maximum change on reproductive success: - Effects on Survival: 0		
Population density	Murine rodent <i>Akodon azarae</i>	Seasonal NDVI	+	Argentina	[92]
Population rate of increase	Kangaroos <i>Macropus sp.</i>	NDVI for 6 months and 12 months	+ (but not better predictor than rainfall)	Australia	[93]

Figure Captions

Figure 1. Results of Functional Principal Component Analysis of the typology of NDVI curves in Idaho, USA, from 1998 – 2011 from April (A) to November (N) for each population-year (dot) identifying two key periods, the spring (2nd FPCA component, the Y-axis) and the fall components (1st FPCA component, X-axis). a) Variation in NDVI curves among populations and years was best explained by FPCA 1, which explained 51.4% of the variation, and characterized primary production from June to October (e.g., summer/fall). b) FPCA 2 (Y-axis) characterized primary production in May and June and explained 27.6% of the seasonal variation. c) NDVI typology was best characterized by 5 clusters, shown in different colors, that corresponded to different patterns of spring and fall primary production, compared to the mean NDVI curve across all of Idaho. For example, typology 5 was characterized by low NDVI intensity in both spring and fall, typology 2 by high NDVI intensity in both spring and fall, and typology 4 by high NDVI intensity in spring, but low in fall, etc.

Figure 2. Distribution of the 5 NDVI typologies shown in Figure 1, with corresponding colors (inset) across the 13 mule deer populations (GMU's) in Idaho, USA, from 1998 - 2011. The size of the pie wedge is proportional to the frequency of occurrence of each NDVI typology within that mule deer population. For example, population 56 had all but one population-year occurring in NDVI typology 4 (Fig. 1) indicating low primary productivity during spring but higher during fall.

Figure 3. Hierarchical Bayesian Path Analysis of the effects of spring and fall growing season functional components (from Fig. 1) and winter precipitation on mule deer fawn body mass and overwinter survival from 1998 – 2011 in Idaho, USA. This model explained 44.5% of the variation in survival. Beta coefficients and their standard deviation are shown, with solid lines indicating the indirect effects of NDVI on survival mass through their effects on body mass, and dashed lines indicate the direct effects of NDVI on survival.

Figure 4. Results of hierarchical Bayesian path analysis showing the standardized direct effects of a) FPCA component 1 from the functional analysis (Fall NDVI), and b) FPCA component 2 (Spring NDVI) on body mass (kg) mule deer fawns in Idaho, USA, from 1998 – 2011.

Figure 5. Results of hierarchical Bayesian path analysis showing standardized direct effects of a) body mass (kg), b) cumulative winter precipitation (in mm), c) FPCA component 1 from the functional analysis (Fall NDVI), and d) FPCA component 2 (Spring NDVI) on the overwinter survival of mule deer fawns in Idaho, USA, from 1998 – 2011.

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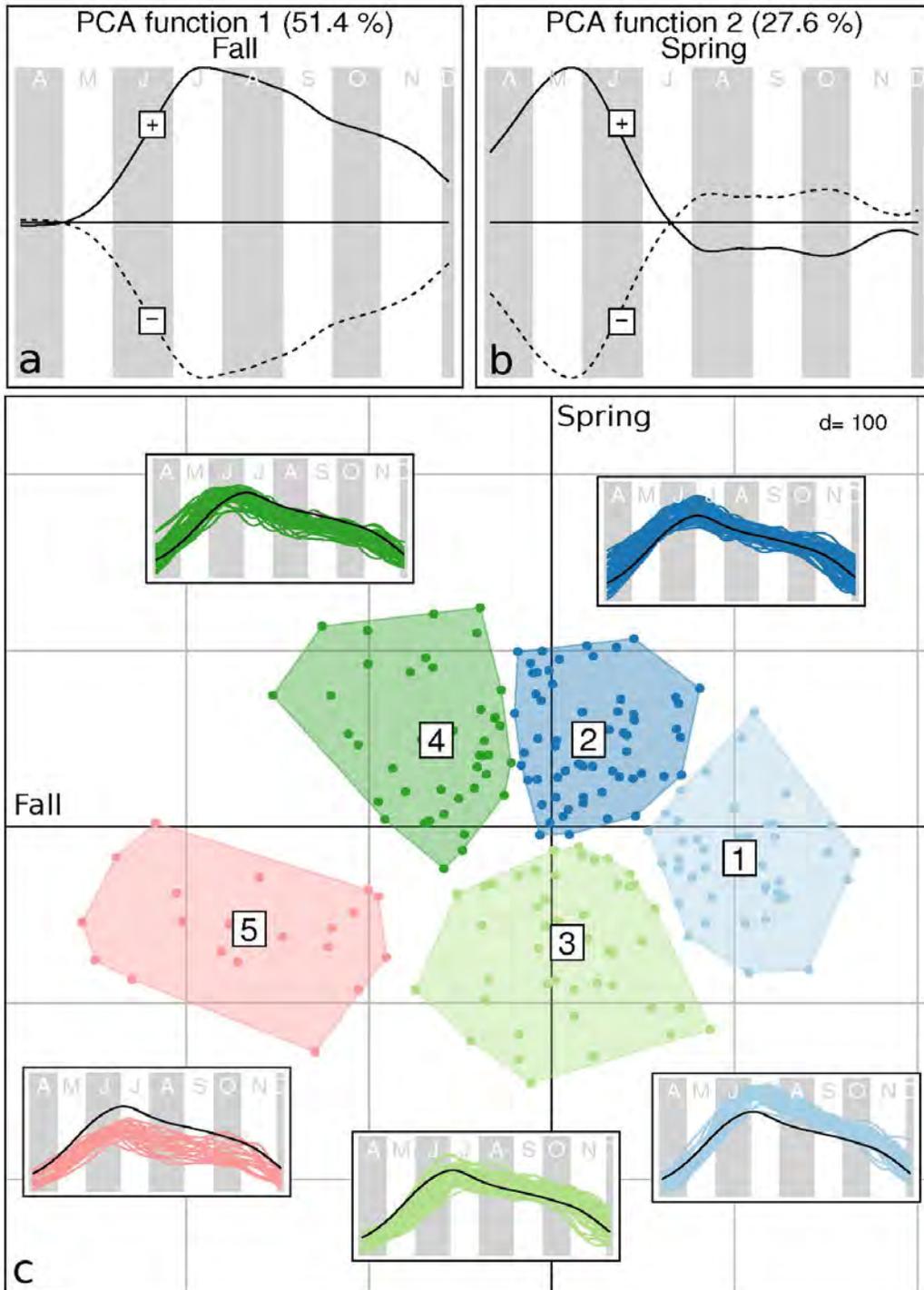


Figure 1

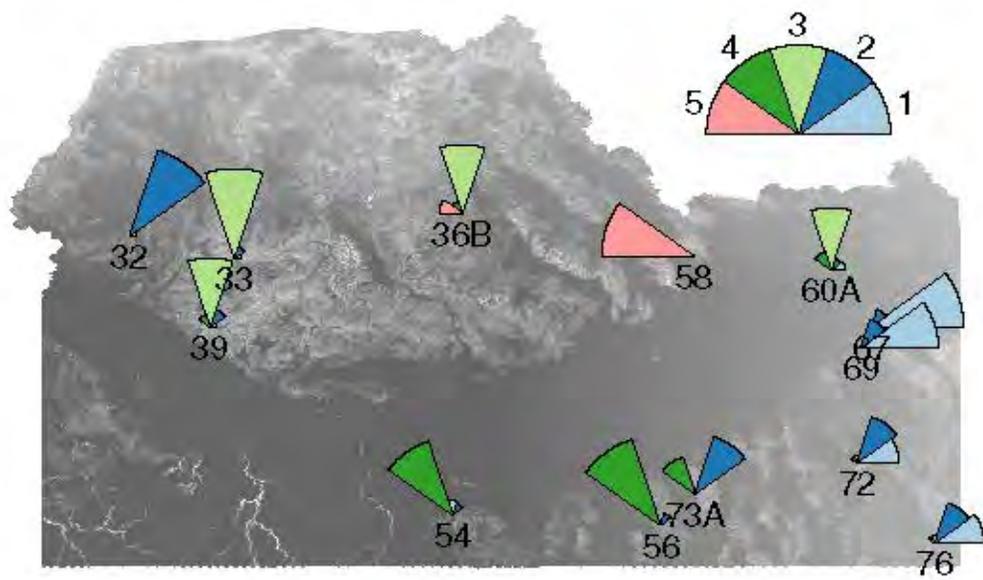


Figure 2

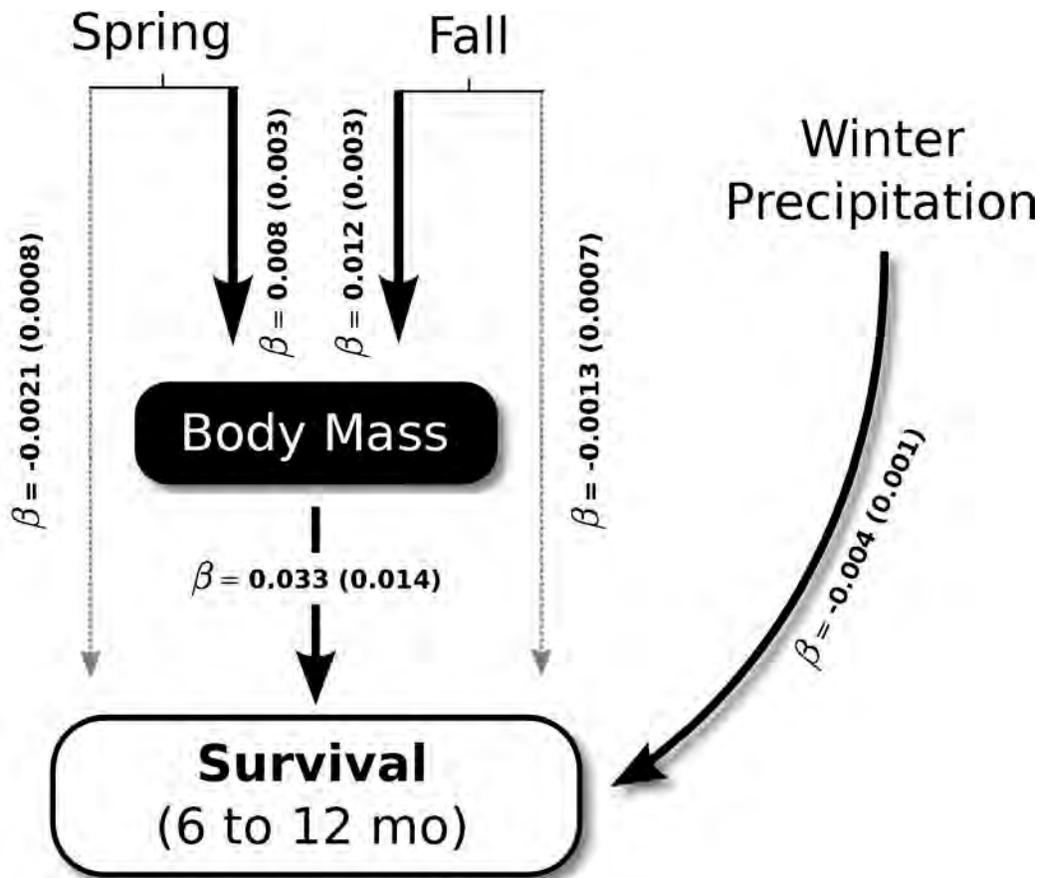


Figure 3

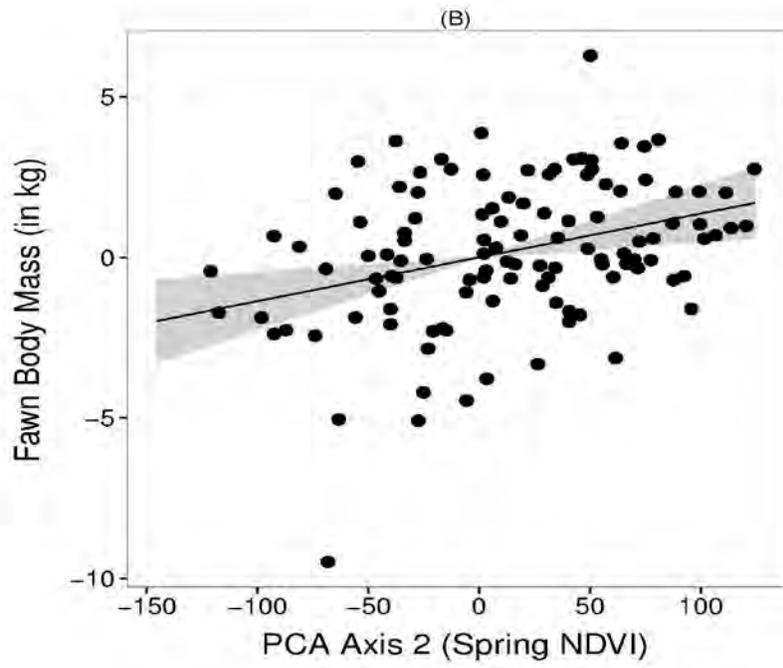
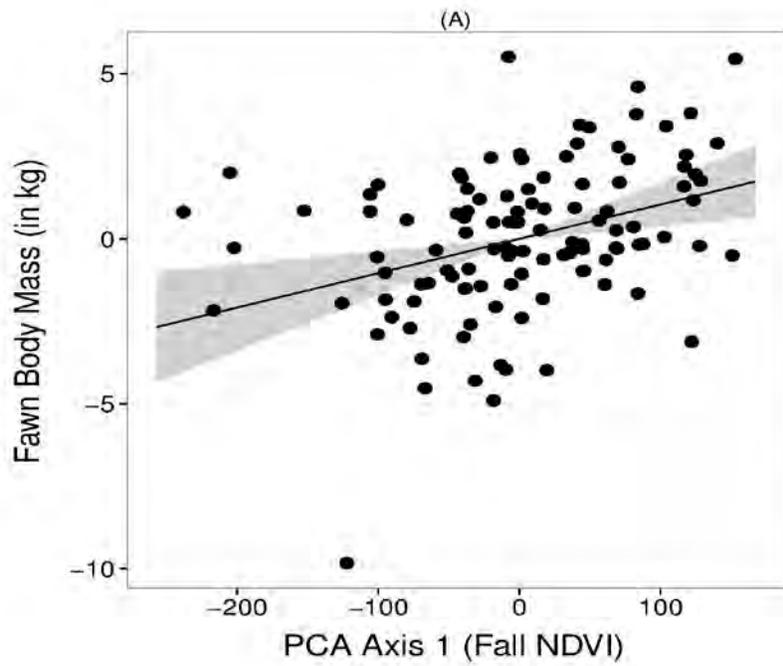


Figure 4

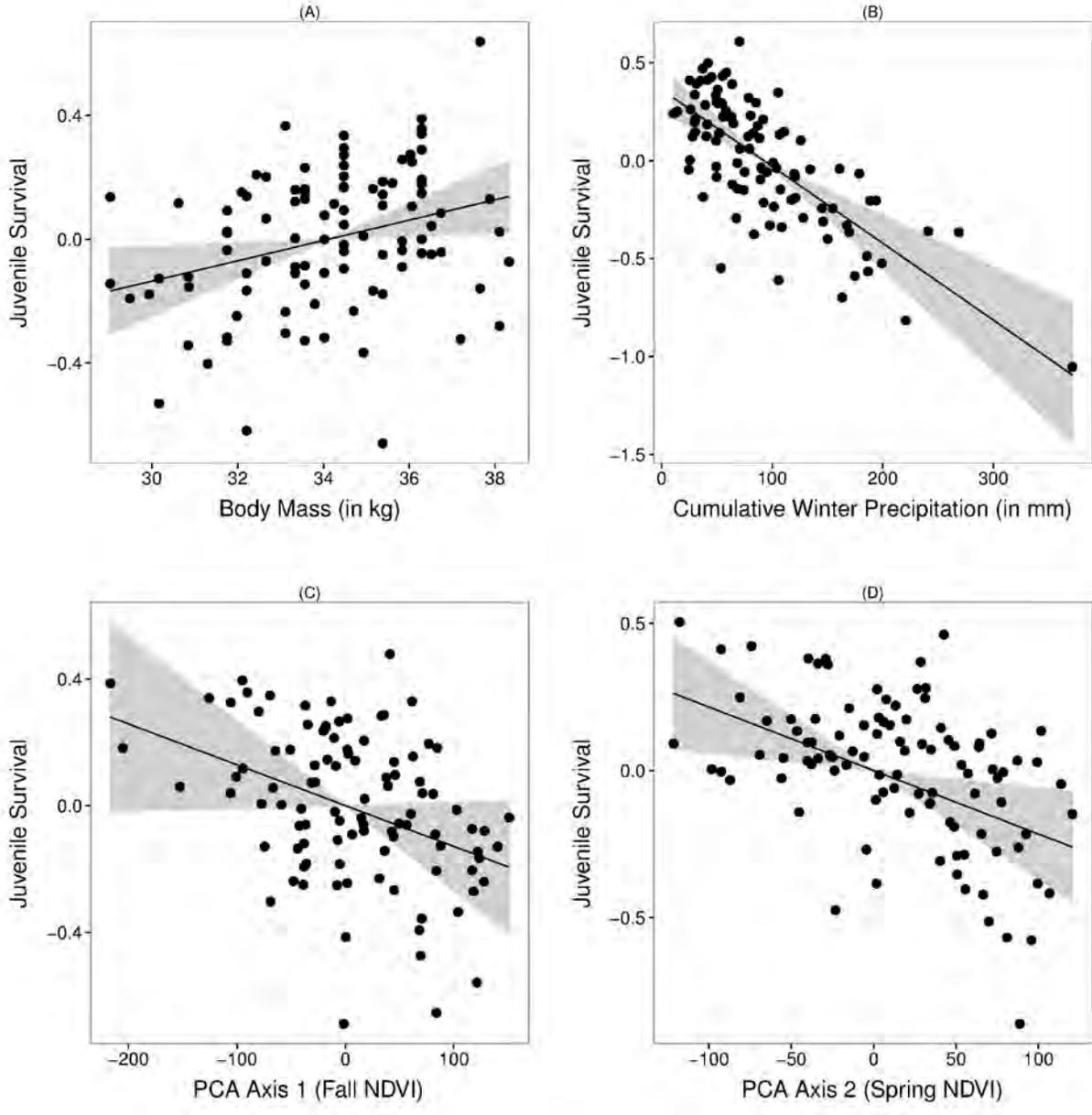


Figure 5

STUDY I, Job II: Estimate the effect of habitat quality of summer range on survival and recruitment of fawns.

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ABSTRACT: Weather conditions and plant composition will determine the nutritional quality of mule deer summer ranges. Nutrition of the adult female and fawns has been shown to influence the growth rate of fawns, a quality strongly related to winter survival. To link nutritional quality to mule deer vital rates, we measured seasonal forage quality and plant composition of adult female summer ranges. Forty-seven phenology plots were initiated in May and June, 2012 in all 3 of the major ecotypes in southern Idaho and revisited 157 times in 2012 and 139 in 2013 during mid to late summer. We collected plant samples at each phenological stage for analysis of crude protein and digestible energy. We will then link actual measured nutritional quality of a vegetation type to remotely sensed measures of NDVI to produce a broad scale estimate of seasonal habitat quality. Plant cameras were deployed at phenology plots as another intermediate step to link RGB levels of the photograph (n = 48,000) to the MODIS spectral signature. To understand how summer range selection influence fawn survival, we estimated plant composition of 38 home ranges of adult females with GPS collars distributed across the ecotypes in 2013 (18 in 2012). We averaged 5 transects per home range to adequately characterize the vegetation composition for each deer (85 transects in 2012 and 196 in 2013). Fecal samples were collected in each home range to estimate diet composition and selection. The summer range specific, nutritional quality estimates will be backcast using the NDVI values to include the years 1998-2013 for which empirical estimates of mule deer fawn survival and fawn ratios are available. Relationships of these vital rates to summer range nutritional quality will be modeled to determine the effect on mule deer population dynamics. To test the importance of different vital rates to population growth, we have developed an integrated population model and are testing it for functionality. Inputs for this model include; age and sex ratios, age specific survival, harvest, and intermittent population estimates. We added modules for estimating survival and visibility bias in aerial surveys in 2013.

INTRODUCTION

Preliminary results from job 1 indicate weather and remotely sensed measures of habitat quality are insufficient to predict population trends in all population management units. Although these metrics may predict well in some PMUs, modeled predictions of winter fawns survival may vary

widely from observed survival in other PMUs. This result may be partially explained by plant community structure. Vastly differing plant communities may have similar NDVI values, as this metric provides a general measure of photosynthetic activity and is not directly correlated to forage quality (e.g. invasive vs. native plant communities). The relationship between the broad scale metrics, precipitation and NDVI, must be validated with known measures of plant nutritional composition to be a reliable measure of forage quality. Once validated, NDVI and weather conditions will become a valuable index across large spatial and temporal extents.

This ability to assess spatial and seasonal distribution of nutrition will become critical for estimating survival and population growth of mule deer within various ecotypes in Idaho. Nutrition affects juvenile survival, age of first reproduction, pregnancy, birth mass and adult survival (Bender et al. 2007, Bishop et al. 2009, Tollefson et al. 2010). Lactation is the most nutritionally demanding part of the annual nutritional cycle (Sadleir 1982), leading researchers to conclude that summer nutrition may be more important to population growth than winter nutrition (Cook et al. 2004). When nutrition is partitioned into the two basic components, energy is critical for survival whereas protein obtained during summer is required for reproduction (Parker et al. 2009). Nutrition during winter (energy) minimizes body fat loss, but rarely changes the importance of growing season nutrition (protein) because the primary factor in winter is expenditure of energy which was stored during summer. For example, Tollefson et al. (2010) recently investigated the effects of nutrition on mule deer production and concluded that summer forage quality has the greatest impact on mule deer populations. However, the mechanistic link between nutrition from summer forage and population dynamics is lacking, limiting our ability to predict population responses to environmental variation. An enhanced understanding of the relationship between estimates of forage quality of a community, assimilated nutrition, and resulting vital rates will provide that link.

Forage quality for herbivores is manifested by a combination of plant occurrence and plant phenology (Parker et al. 2009, Hebblewhite et al. 2008). Plant species within a community have an inherent difference in forage quality for forage depending on growth type with herbaceous understory plants (graminoid, forbs) generally higher quality than woody vegetation (e.g., Hebblewhite et al. 2008). Ungulate forage quality is a function of both abundance and, more importantly, digestibility (Van Soest 1982, Parker et al. 2009). We will focus on summer forage quality because nutrient during this period is likely the primary determinant of fawn survival, adult survival and production (Cook et al. 2004, Parker et al. 2009). The abundance of summer forage quality will dictate the ultimate nutritional carrying capacity of a particular landscape (Wallmo et al. 1977). Recent studies have successfully examined landscape-scale nutritional ecology of ungulates using new remote sensing tools to link forage quality, phenology and landcover (Myysterud et al. 2001, Pettorelli et al. 2007, Hebblewhite et al. 2008, van Beest et al. 2010).

Therefore, in this job, we will develop landscape models for summer forage quality to define potential habitat quality of mule deer in summer and winter. We will then link seasonally and annually varying forage quality to remotely sensed measures of phenology to estimate the effect of changes in available nutrition on mule deer vital rates (survival and productivity).

METHODS

We will include habitat quality to increase the predictive ability of survival models where weather conditions alone are inadequate as covariates. We will determine forage quality at 2 scales, first the individual level and then the PMU. We will use previously collared deer data to estimate kernel density home range size and shape in each of the 3 represented ecotypes and then apply these home range polygons to a random sample, stratified by ecotype, of 150 VHF collared adult females. Availability and quality of forage will move up through spatial resolution from the very fine scale individual plant to estimation of annual variation with freely available remote sensed imagery.

We mapped detailed forage species within fawn rearing ranges in the aspen, shrub/steppe and conifer ecotypes of eastern Idaho. The sampling methods for this work include a detailed estimation of availability within an adult female fawn rearing home range. Seven to ten 100 m point intercept transects will be completed for each of 200 adult female home ranges each year. These samples will form the lowest level of the plant species modeling of the higher hierarchical levels. Fresh mule deer fecal samples from the focal collared animals will be collected for microhistological analysis to determine forage species use by the adult female. Individual plant species frequently selected by deer will be collected during plot sampling and analyzed for digestible energy (DE), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and total percent dry matter digestibility %(DMD); with nutrition adjusted for secondary compounds (Freeland and Janzen 1974, Hanley et al. 1992, Villalba et al. 2002, Baraza et al. 2005). Adult female home ranges will be ranked in nutritional quality by the digestibility, energy, and protein content of the plants used versus available plants.

To scale up to the remote sensing level, we will produce a fine scale model map of forage quality. Using the fine scale vegetation plot data, a forage composition and ground cover model will be constructed using high resolution imagery. An abbreviated sampling scheme (focused on most selected plants) to capture plant composition will be used to ground truth the forage quality map within randomly selected adult female home ranges. One final scaling is required to move the forage quality map to freely available remote sensing imagery. A combination of Aster and spot has promise to provide a high resolution vegetation map of predicted plant compositions at a 15 m resolution. We will perform a supervised classification of the combined images using the previously produced forage model as the training image.

Phenology was determined at approximately 2 week intervals [16 day Moderate Resolution Imaging Spectroradiometer (MODIS)] for representative community types at the 2 extreme elevation gradients in which these species communities occur (July through August). Because we require fine spatial resolution, MODIS data will be used to calculate the Enhanced Vegetation Index (EVI) and NDVI prior to MODIS availability in 2000. Vegetation phenology plots consisted of 10 nested species composition plots along a 100m transect stratified by elevation, community type, and aspect. Plant cameras were deployed at plots to document the daily growth cycle of plants within each MODIS window with plant samples collected for nutritional analyses. Plots were revisited and re-estimated on approximately a 4-week cycle from May through September. Plants frequently used by mule deer were collected and

analyzed for quality change to link EVI data to plant phenology stage and resulting availability. The primary function of interest is the rate of quality decline from growth initiation to desiccation. The final product of this work will include a vegetation composition and quality map of mule deer summer/fall range that can be adjusted annually for quality using EVI derived data from MODIS.

Survival models developed for primary ecotypes in Idaho will be validated against survival estimates of individual PMUs to determine the model that best predicts fawn and adult survival specific to that PMU. The existing ecotype models will be modified with forage quality data to reflect PMU heterogeneity. Once PMU survival models are completed, they will be incorporated into a population model based on survival, fawn ratios, and 4-year interval population surveys (Idaho Mule deer Management Plan 2008). Integrated population models (Johnson et al. 2010) will be constructed with an R base, incorporating an interface for data entry and predictive modeling scenarios. These Bayesian state-space models allow for the combination of several metrics (i.e. survival, fawn ratios, population estimates) with inherently varying data quality. The final produce will include a detailed manual including instructions for spatial data acquisition and model usage.

RESULTS

Vegetation classification and phenology

Vegetation sampling was our primary fieldwork component for this reporting period. Forty-seven phenology plots were initiated in May and June, 2012 in all 3 of the major ecotypes in southern Idaho and revisited 157 times in 2012 and 139 in 2013 during mid to late summer (Table 1). The plots were placed within mule deer summer range in homogeneous vegetation types large enough to contain a single MODIS pixel to facilitate linking vegetation phenology to the MODIS value for that area. Plant cameras were deployed at 70% of the transects in 2012 as another intermediate step to link RGB levels of the photograph to the MODIS spectral signature. In 2013, we concentrated the cameras on the flat open sites (4-6 cameras/site) to maximize the likelihood of a season long stream of daily photos. We collected plant samples for nutritional analysis and each phenological stage of maturity and location.

We estimated plant composition of 38 home ranges of adult females with GPS collars distributed across the ecotypes (Table 1). Point intercept and line transects were conducted in each of the vegetation types contained within a home range to inform high-resolution SPOT imagery. We averaged 5 transects per home range to adequately characterize the vegetation composition for each deer (85 transects in 2012 and 196 in 2013).

Integrated population models

To test the importance of different vital rates to population growth, we have developed an integrated population model and are testing it for functionality. Inputs for this model include; age and sex ratios, age specific survival, harvest, and intermittent population estimates. We added modules for estimating survival and visibility bias in aerial surveys in 2013. An R-based interface is currently under design to input data and extract data from databases to estimate population size with the IPM. This interface will include the final survival models described in the previous section. The IPM will be finalized in 2014.

Table 1. Phenology and composition plots conducted during summer 2013.

Ecotype	GMU	Composition Plots		Phenology plots by stand type							
		Home ranges	Transects	Open flat		Open south slope		Coniferous forest		Aspen forest	
				Plots	Visits	Plots	Visits	Plots	Visits	Plots	Visits
Aspen	56	1	4	2	4	1	4	0	0	1	4
Aspen	73A	1	6	0	0	0	0	0	0	0	0
Aspen	65	0	0	0	0	1	4	1	4	1	4
Aspen	66	11	61	0	0	0	0	0	0	0	0
Aspen	76	1	4	1	4	1	4	1	4	1	4
Conifer	22/32A	3	13	1	4	1	4	1	4	0	0
Conifer	23/24	0	0	1	4	0	0	2	4	0	0
Conifer	36	1	4	2	4	0	0	2	4	0	0
Conifer	36B	4	27	1	4	1	4	1	4	0	0
Conifer	39	3	13	2	4	1	4	3	3	0	0
Conifer	43	1	4	0	0	0	0	0	0	0	0
Conifer	60A/62A	2	9	2	4	0	0	1	4	1	4
Shrub	29/30/30A	3	12	0	0	0	0	0	0	0	0
Shrub	49/50	3	18	2	4	1	4	1	4	1	4
Shrub	54	3	15	2	4	0	0	0	0	1	4
Shrub	59/59A	1	6	2	4	2	4	1	4	0	0
Totals		38	196	18	44	9	32	14	39	6	24

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STUDY II: EFFECTS OF WOLF PREDATION ON ELK AND MOOSE POPULATIONS

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ABSTRACT: During FY13, we captured 31 elk including 11 cows, and 3 bulls, and 17 calves in the North Fork study area. Two elk were recaptured in the Lowman study area to remove GPS collars and download location data. Elk in the North Fork were fitted with GPS radio collars and we obtained various measurements and biological samples. We measured rump fat on captured elk either by palpation or a combination of palpation and ultrasonography. Cow elk serum was evaluated for levels of PSPB to determine pregnancy. We extracted a canine tooth from all elk >3.5 years age and teeth were sectioned to determine age. Elk fecal samples were examined to determine the presence of giant liver fluke ova and all were negative. We captured 13 wolves in the North Fork that were fitted with GPS-Argos radio collars. We conducted monitoring flights on a biweekly basis to determine survival status of elk and wolves. We conducted necropsies on 15 elk and 5 wolves. We downloaded location data from GPS collars obtained from dead animals and collars recovered following release. We currently have 56,532 wolf GPS locations and 560,080 elk GPS locations. We conducted aerial surveys in the North Fork during March to measure the spatial distribution and abundance of elk and alternate ungulate prey. We measured snow depth and sinking depth at 5 sites. We continue to populate an Access database with data from captures, monitoring flights, necropsies, and GPS data from collars.

INTRODUCTION

There is considerable debate over the influences of wolf predation on elk population dynamics. Much of the currently available information comes from work done in various studies on the northern Yellowstone herd. The effect of wolves on northern Yellowstone elk is intensely debated among scientists and resource managers with some (Eberhardt et al. 2003, White and Garrot 2005) describing more substantial effects than others (Smith et al. 2003, 2004; Wright et al. 2006). Because wolves often select calves, senescent elk, or elk with poorer body condition, researchers have speculated that wolf mortality might be largely compensatory (Smith et al. 2003, 2004). However, wolf predation on prime age elk and elk in “good” condition can be substantial (Kunkel and Pletscher 1999, White and Garrott 2005). Calf:cow ratios typically decline appreciably in the presence of wolf caused mortality, and low recruitment is a driving force of population decline (Raithel et al. 2007). With the reestablishment of wolves in the northern Rocky Mountains, the addition of wolf predation caused reduced elk survival rates (Kunkel and Pletscher 1999), lower population growth rates (Hebblewhite et al. 2002, Garrott et al. 2005), lower calf recruitment (White and Garrott 2005), and elk abundance declines (Kunkel

and Pletscher 1999, Garrott et al. 2005), in some herds. Additionally, the effect of wolf predation on ungulate demographics can be exacerbated in the presence of additional large carnivores (Messier 1994, Van Ballenberghe and Ballard 1994).

Wolf kill rates (number of prey or mass of prey killed/wolf/unit time) tend to be somewhat constant over wide ranges of prey densities (Eberhardt 1997, 2000; Eberhardt and Petersen 1999; Boutin 1992; Smith et al. 2004; Garrott et al. 2005; although see Hebblewhite et al. 2003), declining only at very low prey densities (Eberhardt 1997, 2000). This suggests wolf kill rates can be largely independent of prey density. Alternatively, wolves have responded to changes in prey density functionally or numerically in a density dependent fashion (type II functional response) (Dale et al. 1994, Messier 1994). At present, the regulatory (density dependent) nature of wolf predation is unclear (Boutin 1992, Messier 1994, Van Ballenberghe and Ballard 1994).

Although wolf kill rates tend to be somewhat constant, rates of wolf caused mortality often vary widely spatially and temporally, even within individual study areas (Garrott et al. 2005). Consequently, the impacts of wolf caused elk mortality cannot be generalized. The variation largely may be a function of differences in relative densities of elk and wolves. When present, elk are often the preferred prey of wolves (Carbyn 1983, Huggard 1993, Hebblewhite et al. 2003, Garrott et al. 2005), and kill rates are generally a direct function of the prey biomass required by wolves (Thurber and Peterson 1993, Dale et al. 1994, Ballard et al. 1997, Carbyn 1983). Additionally, wolf predation on elk is potentially influenced by other covariates including the relative abundance and vulnerability of alternate prey (Carbyn 1983, Kunkel et al. 1999), the presence of other large predators (Messier 1994, Van Ballenberghe and Ballard 1994, Atwood et al. 2007), habitat characteristics (Kunkel et al. 2004, Creel and Winnie 2005, Mao et al. 2005, Bergman et al. 2006, Atwood et al. 2007), season (Smith et al. 2004), and winter weather severity, especially related to snow accumulation (Huggard 1993, Jaffe 2001, Mech et al. 2001, Hebblewhite et al. 2002, Smith et al. 2004). Wolves tend to selectively prey on calves (Carbyn 1983, Husseman et al. 2003, Smith et al. 2004, Creel and Winnie 2005, Wright et al. 2006), older and senescent elk (Carbyn 1983, Kunkel et al. 1999, Atwood et al. 2007, Wright et al. 2006, Eberhardt et al. 2003), and may select adult bulls over adult cows (Hamlin 2006, Atwood et al. 2007). Thus, the relative abundance of various sex, age, and condition classes of elk might influence predation rates on other classes, in much the same manner as alternate prey.

We will model wolf-caused mortality of elk (wolf caused mortality rates) as a function of wolf abundance, relative to elk abundance (wolf-days/elk). To an extent, this approach assumes density independent predation impacts (Gasaway et al. 1983), whereas the impact of wolf predation on survival might vary with elk density (Messier 1994), because the ratio relationship ignores functional responses of wolves (Messier and Crete 1985). Beyond the primary relationship between wolf-days/elk and survival, we will attempt to explain additional variability with other covariates such as snow and alternate prey densities. This work will provide a basis for measuring the additivity of wolf predation and will provide a tool for predicting wolf caused elk mortality. This will mirror similar work previously conducted on moose-wolf systems (Messier 1994, Eberhart et al. 2003) and it will further preliminary work on elk-wolf systems (Eberhardt et al. 2003). Our findings will have immediate application for wolf and elk managers in Idaho and elsewhere.

We have the following predictions: 1) Wolf caused mortality of elk will increase with increasing wolf-days/elk; 2) The abundance of alternate prey biomass, relative to wolf-days, will dilute the effect of wolf predation; 3) Wolf caused mortality will increase with increasing snow accumulation; 4) The risk of detection, and conditional risk of elk mortality will vary with topography and vegetation composition (*sensu* Hebblewhite and Merrill 2007), and 5) Wolf caused mortality will vary seasonally.

STUDY AREA

We conducted this work on 2 study areas in Idaho. The North Fork of the Clearwater River study area (North Fork) in north central Idaho included the upper reaches of the North Fork of the Clearwater River drainage and the Kelly Creek drainage in Game Management Unit (GMU) 10. The area was characterized by steep, rugged, mountainous terrain ranging in elevation from 650m to 2,400m. Forest vegetation types ranged from ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*) types on xeric sites to western red cedar (*Thuja plicata*) types on mesic sites. Higher elevations included Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) forest stands. Upland meadows were common throughout the area. Many of the south exposures at low elevations were dominated by extensive shrub-fields that resulted from large wildfires in the early 1900s. The Lowman study area in southwest Idaho included the upper reaches of the South Fork of the Payette River drainage in GMUs 33, 34, and 35. The study area was mountainous and ranged in elevation from 900m to 2,700m. Vegetation associations were drier than those on the North Fork study area. Most of the lower elevation habitats along the South Fork of the Payette River included seral shrub-fields and meadows resulting from an extensive wildfire in 1989.

METHODS

We captured and radio-collared adult male and female elk, and calf elk during winter via helicopter darting, ground darting, and in a corral trap. Darted elk were immobilized with Carfentanil and reversed with Naltrexone. We fitted elk with VHF and GPS telemetry collars (Telonics, Mesa, Arizona; Lotek, Newmarket, Ontario, Canada; Vectronic Aerospace, Berlin, Germany; Telemetry Solutions, Concord, California). Elk were monitored with fixed-wing flights biweekly to detect mortality signals. If a mortality signal was detected, the carcass was investigated to determine cause of death as described by Hamlin et al. (1984). Blood samples were drawn from each adult female for pregnancy testing, with analysis for Pregnancy Specific Protein-B (Biotracking, Moscow, Idaho; Noyes et al. 1997). Chest girth was measured to estimate body weight (Millspaugh and Brundige 1996, Cook et al. 2003). Elk <3.5 years of age were aged via tooth wear and replacement (Quimby and Gaab 1957). A canine tooth was extracted from anesthetized adults >3.5 years age to determine age via cementum annuli (Matson's Laboratory, Milltown, Montana; Hamlin et al. 2000). Annual sex and age specific survival rates were calculated following Pollock et al. (1989). We used 2 methods to measure body condition that related directly to fat composition. When practical, we measured maximum rump fat thickness via ultrasonography (Stephenson et al. 1998, Cook et al. 2001a, 2001b). However, this method requires use of an ultrasound device, which was not practical in many

field situations. Alternatively, body fat was evaluated with a body condition score (Gerhart et al. 1996; Cook et al. 2001a, 2001b).

Wolves were captured via leg-hold trapping and helicopter darting. Wolves were immobilized with either Telazol or a combination of Medetomidine and Ketamine. The effects of Medetomidine were reversed with Atipamezol. Wolves were fitted with VHF and GPS telemetry collars, that were capable of data download through the Argos satellite system (Telonics, Mesa, Arizona; Lotek, Newmarket, Ontario, Canada).

We will investigate elk and moose survival as a function of wolf exposure as well as covariates related to weather and habitat conditions. Data analysis will be performed with a model-based approach using Cox's proportional hazards analysis (Hosmer et al. 2008). The required data will include: 1) temporally explicit space use of elk, moose, and wolves 2) dates and locations of elk/moose mortalities and 3) spatially and temporally explicit GIS layers depicting habitat and weather (e.g., snow conditions). All of these parameters vary spatially and temporally across the study sites.

The abundance of elk and ungulate alternate prey (deer, moose) was determined with sightability surveys of study areas (Unsworth et al. 1994). Survey estimates were obtained twice annually corresponding to summer and winter range use. Summer surveys were conducted in late June so that composition ratios could be used to measure early, neonate mortality, and winter surveys were conducted pre-capture during December and January, to estimate additional calf mortality, using composition ratios and estimated cow survival. Those elk abundance estimates were adjusted continuously, post-survey, using age/sex-specific elk survival rates and movements data from radio-marked elk. As elk died or move across the landscape, age and sex-specific densities were adjusted proportionately. The initial resolution was at the level of the survey search units which encompassed 20-60km².

The level and spatial distribution of wolf exposure were determined with GPS-Argos marked wolves. Study area boundaries were defined by wolf pack territories using GPS location data from pack members. Territory boundaries were defined by Brownian bridge movement models (Horne et al. 2007). GPS collars were programmed to obtain a fix every 7.5 hours and Argos downloads occurred every 15 days.

The presence of elk among varying levels of wolf exposure will be determined with GPS locations of radio-collared elk. The majority of elk collars were programmed to obtain locations every 2 hours, during the entire year.

Alternate ungulate prey densities were measured during elk surveys. Moose and mule deer are the dominate alternate ungulate prey and their abundance was estimated using the sightability procedure (Unsworth et al. 1994), during summer and winter study area surveys conducted for elk. Other less abundant ungulate species included mountain goats and white-tailed deer. We indexed white-tailed deer and mountain goat abundance with survey observations.

Winter weather severity, especially related to snow characteristics, can substantially affect wolf caused mortality and forage availability. Snow effects are complex and relate to depth, density, and crusting that all vary with aspect, slope, temperature, etc. Snow water equivalent (SWE), which is a function of depth and density ($SWE/density = \text{snow depth}$), is an often used metric in ungulate research. SWE and depth are typically highly correlated. Sinking depth is potentially more informative than depth alone, and is a function of depth, density, and can be influenced by crusting. Depth alone has been used to predict the influence of snow on wolf predation on elk (Hebblewhite et al. 2002, Huggard 1993). We measured snow accumulation in 2 ways. First, data on depth and SWE are available from SNOTEL sites. The SNOTEL data is available at no cost and is reliable, although the sites are typically at much higher elevations than wintering ungulates. We established permanent snow measuring sites on the Lowman (3 sites) and North Fork (2 sites) study areas. The sites were at low elevation, on southerly slopes and included areas occupied by elk during winter. We measured snow depth and indexed sinking depth with a drop penetrometer, biweekly at each site. This snow condition data will be used to evaluate regional snow cover models (SNODAS) that may be used to extrapolate snow conditions across both study sites.

Our data indicate that malnutrition may be an important mortality factor for elk during some years. It is important, therefore, to understand how forage conditions change throughout the growing season and annually across Idaho's dynamic landscapes. We are exploring the use of Normalized Difference Vegetation Index (NDVI) using remotely sensed data collected via the MODIS satellite system by USGS (eMODIS, Jenkerson et al. 2010) as a means of estimating available forage across the landscapes that are used by ungulates.

RESULTS

On the Lowman study area, adult female elk survival ranged from 0.86 to 0.92 during calendar years 2009-2012 but was 0.74 during 2008 (Table 1). The pattern in adult bull mortality was similar, except that survival was also low in 2010. Low survival during 2008 was attributed to exceptional snow accumulation. Survival of calves captured at 6-months-old averaged 0.35, except during the exceptionally mild winter of 2010, when survival was 0.78 (Table 1).

Both adult and calf survival were substantially higher during 2010 than during 2009 and 2011 in the North Fork study area, as well (Table 1).

Table 1. Annual survival rates of adult elk, and 6-month (capture (approx. Jan) – 31 May) survival rates of calf elk, radio-collared on the North Fork and Lowman study areas in Idaho.

Study Area	Bulls		Cows		Calves	
	S ^a	(SE)	S	(SE)	S	(SE)
Lowman						
2008	0.42	(0.08)	0.74	(0.05)	0.35	(0.06)
2009	0.65	(0.09)	0.92	(0.04)	0.30	(0.06)
2010	0.47	(0.08)	0.87	(0.04)	0.78	(0.09)
2011	0.75	(0.11)	0.86	(0.05)	0.40	(0.11)
2012	0.85	(0.32)	0.90	(0.06)	NA	
North Fork						
2009	0.71	(0.10)	0.79	(0.06)	0.09	(0.03)
2010	0.88	(0.06)	0.95	(0.03)	0.60	(0.10)
2011	0.71	(0.09)	0.80	(0.06)	0.23	(0.10)
2012	0.82	(0.10)	0.85	(0.06)	NA	

^a Survival rates and standard errors calculated following Pollock et al. (1989).

On the Lowman study area wolf predation was the primary source of mortality (Table 2). Malnutrition alone was also a significant source of mortality (Table 2). Most deaths associated with malnutrition occurred during the winter of 2008, when snow accumulation was substantial. The primary cause of bull elk mortality was hunter harvest.

Table 2. Cause-specific mortality of radio-collared elk on the Lowman study area in Idaho, 2008-2012 (calendar year); expressed as a percentage of mortalities (*n*) in which cause-of-death could be assigned based on available evidence.

Cause of Death	Bulls (<i>n</i> = 36)	Cows (<i>n</i> = 20)	Calves (<i>n</i> = 36)
Wolf	25%	35%	44%
Wolf/malnutrition ^a	6%	10%	31%
Cougar	0%	10%	6%
Predation, species unknown	3%	0%	3%
Malnutrition	6%	20%	11
Natural accident	3%	0%	3%
Auto accident	0%	0%	3%
Legal hunter harvest	58%	20%	0%
Illegal hunter harvest	0%	0%	0%
Old age	0%	5%	0%

^a Necropsy revealed that the death was caused by predation, but malnutrition was indicated by mostly or entirely depleted bone marrow fat.

On the North Fork study area, wolf predation was the primary source of mortality (Table 3). Pregnancy rates for adult cow elk varied considerably (Table 4), although sample sizes were insufficient to draw any solid conclusions.

Table 3. Causes of death determined from necropsies of dead radio-collared elk on the North Fork study area in Idaho, 2009-2012 (calendar year).

Cause of Death	Bulls (<i>n</i> = 10)	Cows (<i>n</i> = 8)	Calves (<i>n</i> = 27)
Wolf	60%	63%	85%
Wolf/malnutrition ^a	0%	13%	4%
Cougar	10%	13%	7%
Predation, species unknown	0%	0%	4%
Malnutrition	0%	0%	0%
Natural accident	0%	0%	0%
Auto accident	0%	0%	0%
Legal hunter harvest	30%	0%	0%
Illegal hunter harvest	0%	13%	0%
Old age	0%	0%	0%

^a Necropsy revealed that the death was caused by predation, but malnutrition was indicated by mostly or entirely depleted bone marrow fat.

Table 4. Pregnancy rates of adult (2 year +) elk captured on the Lowman and North Fork study areas in Idaho during Jan or Feb of indicated year.

Study Area	Lowman	North Fork
	% Pregnant ^a (n)	% Pregnant (n)
2008	77 (24)	
2009	80 (4)	100 (9)
2010	67 (18)	75 (12)
2011	100 (7)	0 (1)
2012	38 (8)	100 (1)

^a Pregnancy determined from levels of Pregnancy Specific Protein-B following Noyes et al. (1997).

Limitations imposed by helicopter and capture crew availability, and project personnel turn-over resulted in a reduced capture effort in January–February 2012 compared to previous years. This was most evident in the lack of 6-month-old calves, the cohort that typically experiences the highest mortality rate. We maintained monitoring and data collection schedules throughout this period. We began preliminary data analysis, focusing on the Lowman study area, during this reporting period.

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STUDY III: HELLS CANYON AND SALMON RIVER BIGHORN SHEEP

Job I: Hells Canyon Initiative to Restore Rocky Mountain Bighorn Sheep

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ABSTRACT: The Hells Canyon Initiative is a state, federal, and private partnership to restore Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) in the Hells Canyon area of Oregon, Idaho, and Washington. During 2012—2013, 138 radio-collared bighorn sheep were monitored regularly in 14 populations. Average annual survival of radio-collared ewes was 0.84 and annual ram survival was 0.78. Pneumonia-caused mortality of lambs was confirmed in 4 populations and suspected in 5 others. The overall metapopulation was estimated at 780 bighorn sheep, an increase from an estimate of 740 sheep in 2011—2012.

Multi-year collaborative projects were continued with the University of Idaho Caine Veterinary Teaching Center (CVTC) and the Department of Veterinary Microbiology and Pathology at Washington State University to better understand the clinical causes of disease, in particular *Mycoplasma ovipneumoniae*, in bighorn sheep. Three papers were published in peer-reviewed journals.

The goals of the Salmon River bighorn sheep study are to evaluate population and genetic structure of bighorn sheep in the Salmon River drainage in Game Management Units (GMU) 14, 19, 19A, 20, and 20A, and to assess connectivity between these sheep and other Idaho sheep populations. A final report (MS Thesis) is expected by the end of 2013.

KEY WORDS: Bighorn sheep, disease, gene flow, *Mycoplasma ovipneumoniae*, *Ovis canadensis*

The Hells Canyon Initiative was started in 1995 as a program to accelerate restoration of bighorn sheep in Hells Canyon and the surrounding areas of Idaho, Oregon, and Washington and to focus research applicable to bighorn sheep restoration and management throughout the western United States and Canada. The concept was formalized in 1997 with the completion of an interagency memorandum of agreement and restoration plan (Hells Canyon Bighorn Sheep Restoration Committee 1997). The restoration plan was updated in 2004 (Hells Canyon Bighorn Sheep Restoration Committee 2004).

The Salmon River Bighorn Sheep Project was initiated in 2007 and is a collaborative effort between the Nez Perce Tribe, the Idaho Department of Fish and Game, the USDA Forest Service, and the USDI Bureau of Land Management. Data are being collected on bighorn sheep distribution and movements, temporal and spatial use patterns, as well as survival, lamb production and recruitment. The objective of this study is to use genetic tools, habitat, and movement and survival data to investigate connectivity and the risk of contact between and within subpopulations.

STUDY AREA

The Hells Canyon Initiative project area encompasses 2,273,194 ha (5,617,062 ac) in the Snake River drainage in Oregon, Idaho, and Washington from the mouth of Clearwater River, Idaho, south to Brownlee Reservoir. It is bounded on the east by the hydrologic divide between the Salmon and Snake rivers near Riggins, Idaho, south to Brownlee Creek on the Payette National Forest, Idaho, and extends just west of the Eagle Cap Wilderness, Wallowa-Whitman National Forest, Oregon. Major drainages include the Snake, Grande Ronde, Imnaha, and lower Salmon rivers. There are currently 16 bighorn sheep populations, or herds, established in the project area (Fig 1). Over 1.3 million acres (24%) of the project area is potential bighorn sheep habitat, 68% of which is publicly owned, primarily managed by the U.S. Forest Service (USFS). Other public land managers are the states of Oregon, Idaho, and Washington and the Bureau of Land Management (BLM).

Hells Canyon Bighorn Sheep Project Area

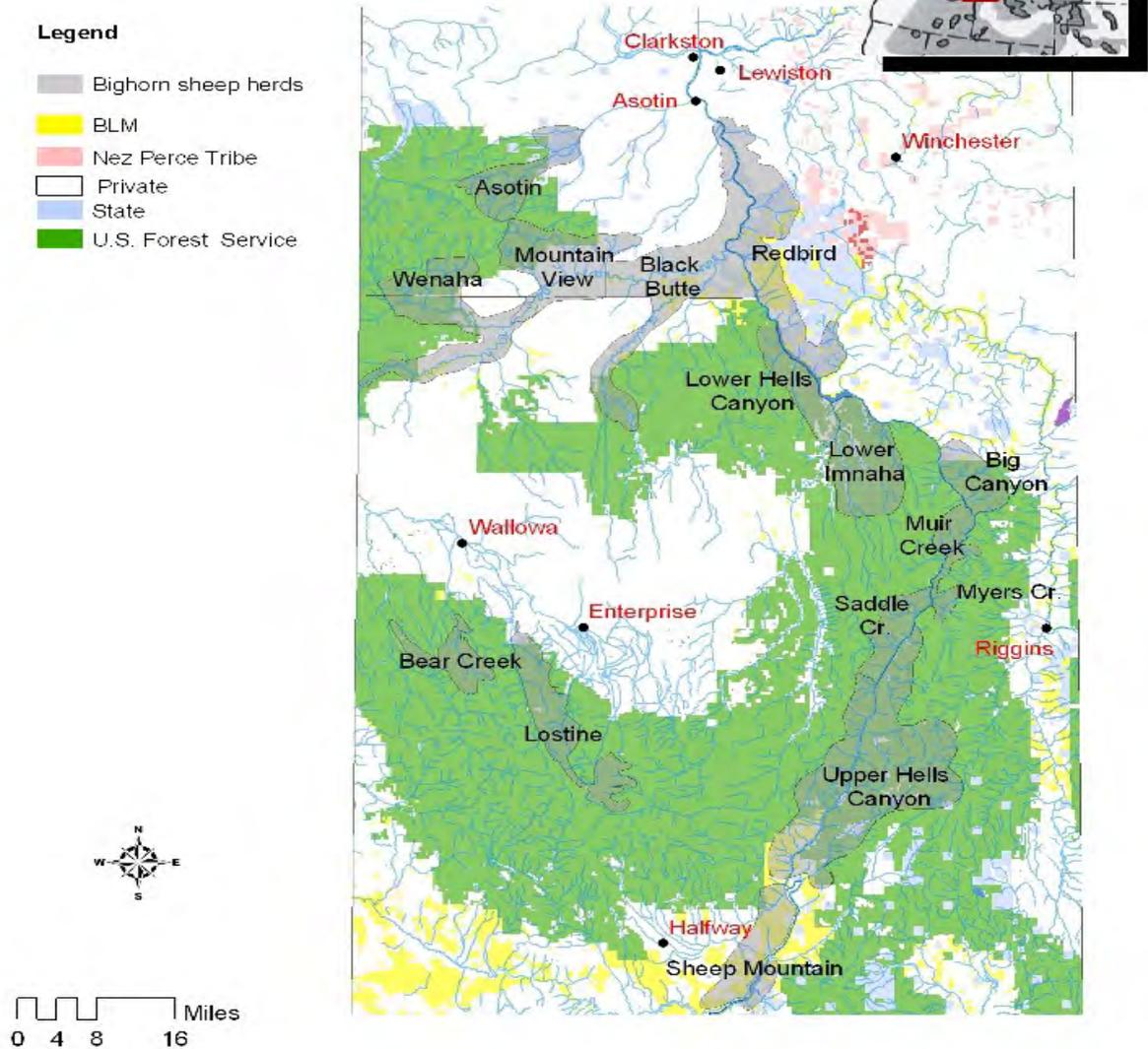


Figure 1. Hells Canyon Initiative Project Area

The greater Salmon River study area includes the portion of Hells Canyon in Idaho, the Salmon River drainage and tributaries downstream from Sunbeam, ID and the Lemhi and Lost River mountain ranges (Fig. 2). Landscapes are rugged and mountainous and elevations range from 245m in river canyons to >3,600m in the Lost River Range. The Salmon River Project focal area (Fig. 3) is contained within the larger study area and includes the Little Salmon River drainage, the mainstem Salmon River upstream from Riggins, ID to Big Mallard creek, the lower part of the South Fork Salmon River, and upper portions of the Payette River drainage.

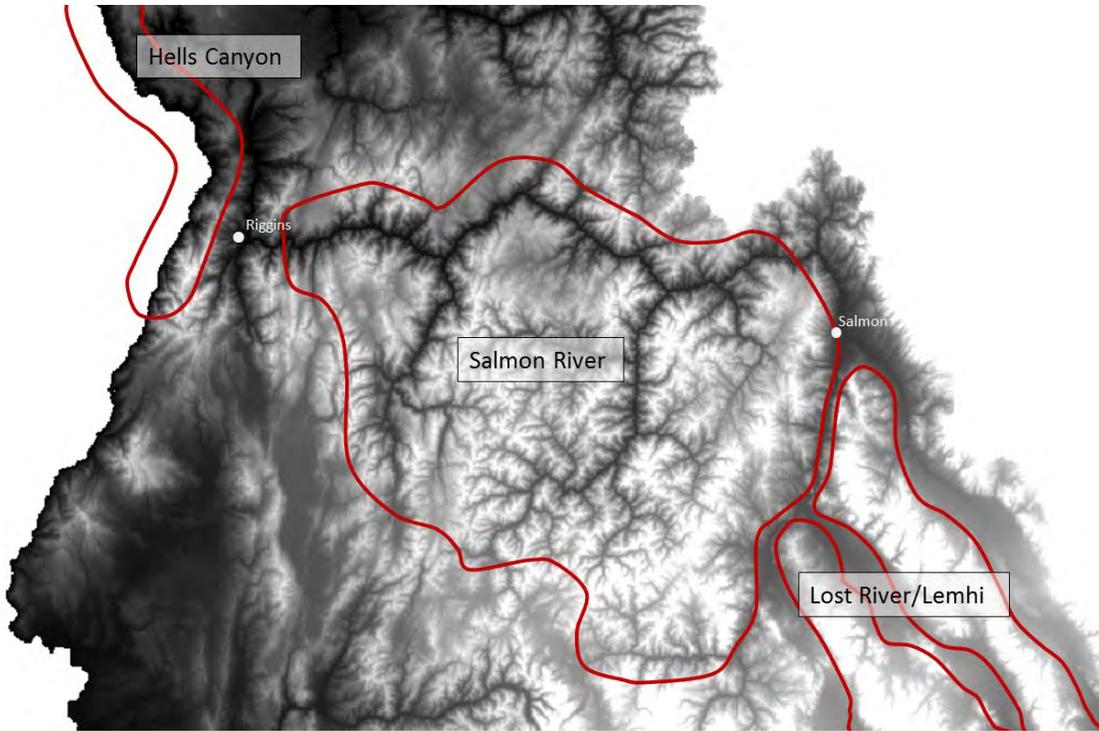


Figure 2. Central Idaho Bighorn sheep subpopulations in the greater Salmon River Study area.

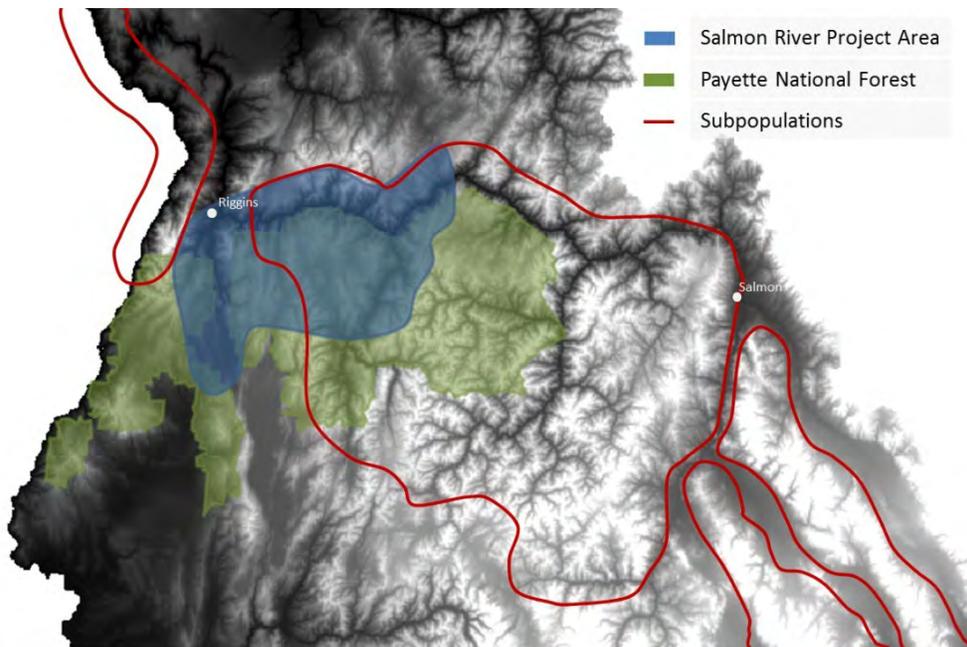


Figure 3. Salmon River Project Area, Payette National Forest, and bighorn sheep subpopulations in central Idaho.

METHODS

Population Monitoring

One hundred thirty-eight radio-collared bighorn sheep (93 ewes, 45 rams) were monitored in 14 Hells Canyon bighorn sheep populations from 1 May 2012 to 30 April 2013. We monitored radio-collared ewes weekly from mid-April through September to determine productivity and as needed to document summer lamb survival and we monitored movements and survival of all sheep a minimum of biweekly year round. We also captured and radio-collared additional bighorn sheep via helicopter netgun and at a corral trap during this reporting period. The state wildlife agencies of Idaho, Oregon, and Washington conducted surveys from a helicopter (Robinson 44 and Hughes 500) and on the ground in 2012—2013 to estimate total population size.

RESULTS AND DISCUSSION

Adult survival

Twenty-five radio-collared adult bighorn sheep (15 ewes, 10 rams) died in Hells Canyon during this period in the Redbird (5), Asotin (3), Lower Hells Canyon (3), Downey Saddle translocation (2), Imnaha (2), Wenaha (2), Saddle Creek (2), Muir Creek (2), Big Canyon, Black Butte, Myers Creek, Upper Hells Canyon Oregon, Sheep Mountain, and Lostine (1 ea) populations. Cause of death could be determined for 7 animals. Known causes of mortality were trauma (3) and harvest (4 rams Asotin (2), Black Butte, Imnaha). Averaged over the metapopulation, annual survival of radio-collared ewes was 0.84 (78/93) and rams was 0.78 (35/45). By population, annual survival of ewes in populations (8) with 5 to 14 radio-collared females ranged from a low in Redbird of 71%, to 100% in Asotin, Lostine, Mountain View, and Black Butte (Tables 1 and 2).

Table 1. Annual ewe survival in 10 Hells Canyon bighorn sheep populations, 1 May 1997 to 30 April 2012.

<i>Year</i>	<i>Black Butte</i>	<i>Redbird</i>	<i>Wenaha</i>	<i>Asotin</i>	<i>Lostine</i>	<i>Imnaha</i>	<i>Big Canyon</i>	<i>Muir Creek</i>	<i>Mountain View</i>	<i>Sheep Mountain</i>
1997-1998	0.92	1.0	0.91							
1998-1999	1.0	1.0	1.0	0.88			1.0	1.0		
1999-2000	0.58	1.0	1.0	0.86			0.93	0.93		
2000-2001	0.71	1.0	0.73	1.0	1.0	0.86	0.57	0.67		
2001-2002	0.80	0.92	1.0	1.0	1.0	1.0	1.0	1.0		
2002-2003	1.0	0.91	1.0	0.80	0.94	1.0	0.88	0.50		
2003-2004	0.84	0.73	0.75	0.83	0.94	0.80	0.29	0.88		
2004-2005	0.75	1.0	1.0	0.80	0.81	0.92	1.0	1.0		
2005-2006	1.0	0.91	0.89	0.88	0.80	0.91	1.0	0.78		
2006-2007	1.0	0.86	0.93	0.73	0.88	1.0	1.0	0.86	1.0	1.0
2007-2008	0.93	0.91	0.62	1.0	0.92	0.80	1.0	1.0	0.60	0.88
2008-2009	0.75	1.0	1.0	1.0	1.0	0.56	0.70	1.0	1.0	0.71
2009-2010	0.83	1.0	1.0	0.83	1.0	0.89	1.0	1.0	0.67	1.0
2010-2011	0.80	1.0	1.0	0.80	0.92	1.0	1.0	1.0	1.0	1.0
2011-2012	0.83	0.82	0.93	0.67	1.0	1.0	1.0	1.0	1.0	1.0
2012-2013	1.0	0.71	0.91	1.0	1.0	0.92	0.70		1.0	0.80
Average	0.86	0.92	0.92	0.87	0.93	0.90	0.87	0.90	0.90	0.91

Table 2. Annual ram survival in 9 Hells Canyon bighorn sheep populations, 1 May 1997 to 30 April 2013.

<i>Year</i>	<i>Black Butte</i>	<i>Redbird</i>	<i>Wenaha</i>	<i>Asotin</i>	<i>Lostine</i>	<i>Imnaha</i>	<i>Big Canyon</i>	<i>Muir Creek</i>	<i>Mountain View</i>
1998-1999							1.0	1.0	
1999-2000							1.0	1.0	
2000-2001	1.0	1.0	0.67		0.80	0.67	0.80	0.50	
2001-2002	0.8	0.80	1.0		1.0	1.0	0.75	0.50	
2002-2003	0.3	0.75	1.0		0.80	1.0	0.33		
2003-2004	0.5	0.83	1.0	0.8	0.80	0.67	1.0	1.0	
2004-2005	1.0	0.60	0.88	0.75	0.83	0.67	1.0	1.0	
2005-2006		1.0	1.0	0.33	1.0	0.50		1.0	
2006-2007	1.0	0.83	0.83	1.0	0.78	0.83			1.0
2007-2008	1.0	0.75	0.71	0.60	1.0	0.63	1.0		0.67
2008-2009	1.0	0.83	0.86	0.92	0.88	0.83			1.0
2009-2010	0.75	1.0	1.0	0.82	0.86	0.60			
2010-2011	0.67	0.60	1.0	0.78	1.0	0.88	1.0	1.0	1.0
2011-2012	1.0	0.82	1.0	0.88	1.0	1.0	1.0	1.0	1.0
2012-2013	1.0	0.75	1.0	0.79	0.83	0.5	1.0		1.0
Average	0.84	0.81	0.92	0.77	0.89	0.75	0.88	0.86	0.95

Lamb Survival

In 2013, summer lamb survival in the 4 populations where lambs were diagnosed with pneumonia was 50% or less (Tables 3, 5), and recruitment in these populations ranged from 12 to 26 lambs/100 ewes (Table 4). Pneumonia-caused lamb mortality was suspected in another 5 populations where summer survival was 0—50% and recruitment was 0 to 24 lambs/100 ewes (Table 4), although no dead lambs were recovered.

Table 3. Observed productivity and summer lamb survival in 9 herds in Hells Canyon, 2012.

Herd	No radio-collared ewes observed with lambs (%)		% summer survival ^a
Asotin Creek, Washington	9/10	(90)	38%
Black Butte, OR/WA	10/11	(91)	50%
Imnaha, Oregon	12/13	(91)	50%
Mtn View/Wenaha, WA OR	17/18	(94)	39%
Muir Creek, OR	2/2	(100)	0%
Redbird, Idaho	7/14	(50)	33%
Lower Hells Canyon, OR	6/7	(86)	0%
Upper Hells Canyon, OR	3/3	(100)	0%
Sheep Mountain, Oregon	0/5	(0)	0%

^a Survival from birth to 1 October. Herds in bold are those where lambs were recovered with pneumonia.

Table 4. Hells Canyon bighorn sheep population counts, 2012—2013.

Herd	Survey date(s)	Total	Ewes	Lambs	Rams	Estimated Population
Asotin, WA ^a	3/2/2013	64	32	5	27	70
Bear Creek, OR	July 2012	47	29	2	16	60
Big Canyon, ID ^a	Mar-Apr 2013	22	10	4	8	25
Black Butte, WA/OR ^a	3/1/2013	52	30	8	14	55
Lostine, OR ^a	Feb 2013	71	36	18	17	75
Lower Hells Canyon, OR ^a	NA	26	16	0	10	35
Lower Imnaha, OR ^a	4/2/2013	109	67	16	26	135
Mtn View/Wenaha, WA/OR ^a	April 2013	108	57	12	39	125
Muir Creek, OR ^a	2/14/2013	16	10	0	6	20
Myers Creek, ID	Dec–Mar 2012	3	2	0	1	5
Redbird, ID ^a	3/9-10/2013	91	63	8	22	95
Saddle Creek, OR	NA	47	28	11	8	55
Sheep Mountain, OR ^a	4/3/2013	10	10	0	0	10
Upper Hells Canyon, ID	12/5/2012	5	2	1	2	5
Upper Hells Canyon, OR	11/2012, 5/2013	8	5	0	3	10
Total		678				780
Average						52

^a Populations monitored intensively under the Hells Canyon Initiative.

Table 5. Dead pneumonic bighorn lambs submitted to the Washington Animal Diagnostic Laboratory 1 May 2012 to 30 April 2013.

<i>Animal ID</i>	<i>WADDL ID</i>	<i>Mortality Date</i>	<i>Herd</i>	<i>Sex</i>	<i>Age (approx days)</i>	<i>Weight (kg)</i>	<i>Mycoplasma ovipneumoniae PCR</i>
12WA01	2012-6349	6/1/2012	Black Butte	M	17	9.8	Pos
12WA02	2012-6349	6/1/2012	Black Butte	M	17	8.9	Po
12WA05	2012-7000	6/7/2012	Black Butte	F	23	11.6	Po
12WA06	2012-7496	6/24/2012	Black Butte	M	40	10.0	Po
12OR04	2012-9613	8/22/2012	Mt. View	F	99	20.9	Po
12ID01	2012-6165	5/29/2012	Redbird	F	14	6.8	Neg
12OR02	2012-7763	7/1/2012	Wenaha	F	47	10.4	Po

Capture

Fifteen bighorn sheep were captured via helicopter net-gunning, radio-collared and released in Asotin Creek in October 2012. We recaptured 6 collared ewes, as well as 2 unmarked ewes and 7 unmarked rams. All sheep were sampled for bacteria, parasites, trace elements and exposure to respiratory pathogens. All sheep were radio-collared with VHF collars and rams were also collared with satellite GPS collars.

Thirty-five bighorn sheep (28 ewes, 4 rams, and 3 lambs) were captured in a corral trap or by immobilization with xylazine at the Lostine winter range and 25 of these were captured more than once to test for *Mycoplasma ovipneumoniae* shedding patterns.

Population Monitoring

Approximately 780 bighorn sheep are estimated to occur in 16 herds or populations within the project area (Table 4). This is an increase in numbers from an estimate of 740 last year. This increase was primarily due to increases in the Saddle Creek, Bear Creek, and Lostine Oregon populations and few declines in other populations. We have incorporated the sightability model we have developed for bighorn sheep in Hells Canyon into the R software program sightability package to better estimate population size and to obtain confidence limits on the estimates.

Disease Research and Management

An intensive analysis of pneumonia dynamics in Hells Canyon bighorn sheep was continued in collaboration with the Center for Infectious Disease Dynamics at Penn State University, Washington State University, and the USGS Rocky Mountain Research Center in Bozeman, MT. Investigation into patterns of Movi shedding in the Lostine population through repeated testing of marked individuals revealed that some individuals tested consistently negative (resistant), others consistently tested positive (chronic shedders), and others switched back and forth (intermittent shedders). This suggests that sheep that survive initial exposure may fall into three categories of Movi infection:

- **Chronic-shedders** become chronically infected and continue to readily transmit Movi.
- **Resistant** individuals clear infection following exposure, and do not subsequently transmit Movi.
- **Incompletely resistant** individuals periodically shed and can transmit Movi, but they also intermittently test negative.

Additional work is planned over the next winter to test this idea.

Three manuscripts were published during this period:

- Besser, T.E., Cassirer, E.F., Highland, M.A., Wolff, P., Justice-Allen, A., Mansfield, K.M., Davis, M.A. & Foreyt, W.J. (2012) Bighorn sheep pneumonia: Sorting out the etiology of a polymicrobial disease. *Journal of Preventive Veterinary Medicine*.
- Cassirer, E.F., Plowright, R.K., Manlove, K.R., Cross, P.C., Dobson, A.P., Potter, K.A. & Hudson, P.J. (2013) Spatio-temporal dynamics of pneumonia in bighorn sheep (*Ovis canadensis*). *Journal of Animal Ecology*, **82**, 518-528.

Plowright, R.K., Manlove, K.R., Cassirer, E.F., Cross, P.C., Besser, T.E. & Hudson, P.J. (2013)
Use of exposure history to identify patterns of immunity to pneumonia in bighorn sheep
(*Ovis canadensis*) population. *PLoS ONE*.

Hells Canyon Bighorn Sheep Restoration Committee

The Hells Canyon Bighorn Sheep Initiative is conducted by the Hells Canyon Bighorn Sheep Restoration Committee. The committee is comprised of state, federal, tribal, and private organizations, of which each provide an administrative (A) and technical (T) committee member. Organizations and committee members in 2012 were:

Idaho Department of Fish and Game

Brad Compton, Wildlife Program Coordinator (A)

Frances Cassirer, Hells Canyon Project Coordinator/Wildlife Research Biologist (T)

Oregon Department of Fish and Wildlife

Nick Myatt, (A)

Pat Matthews, District Wildlife Biologist (T)

Washington Department of Fish and Wildlife

Rich Howad, Big Game Program Manager (A)

Paul Wik, District Wildlife Biologist (T)

USDA Forest Service

Kevin Martin, Forest Supervisor, Umatilla National Forest (A)

Mark Penninger, Full Curl Coordinator, Wallowa-Whitman Forest Biologist (T)

USDI Bureau of Land Management

Tom Rinkes, Wildlife Biologist, Idaho State Office(A)

Craig Johnson, Wildlife Biologist, Salmon-Clearwater Resource Area (T)

Wild Sheep Foundation

Gray Thornton, CEO (A)

Kevin Hurley, Conservation Director (T)

Nez Perce Tribe (Ad hoc member)

Keith Lawrence, Wildlife Biologist (A)

Curt Mack, Wildlife Biologist (T)

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Spatio-temporal dynamics of pneumonia in bighorn sheep

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Summary

1. Bighorn sheep mortality related to pneumonia is a primary factor limiting population recovery across western North America, but management has been constrained by an incomplete understanding of the disease. We analysed patterns of pneumonia-caused mortality over 14 years in 16 interconnected bighorn sheep populations to gain insights into underlying disease processes.

2. We observed four age-structured classes of annual pneumonia mortality patterns: all-age, lamb-only, secondary all-age and adult-only. Although there was considerable variability within classes, overall they differed in persistence within and impact on populations. Years with pneumonia-induced mortality occurring simultaneously across age classes (i.e. all-age) appeared to be a consequence of pathogen invasion into a naïve population and resulted in immediate population declines. Subsequently, low recruitment due to frequent high mortality outbreaks in lambs, probably due to association with chronically infected ewes, posed a significant obstacle to population recovery. Secondary all-age events occurred in previously exposed populations when outbreaks in lambs were followed by lower rates of pneumonia-induced mortality in adults. Infrequent pneumonia events restricted to adults were usually of short duration with low mortality.

3. Acute pneumonia-induced mortality in adults was concentrated in fall and early winter around the breeding season when rams are more mobile and the sexes commingle. In contrast, mortality restricted to lambs peaked in summer when ewes and lambs were concentrated in nursery groups.

4. We detected weak synchrony in adult pneumonia between adjacent populations, but found no evidence for landscape-scale extrinsic variables as drivers of disease.

5. We demonstrate that there was a >60% probability of a disease event each year following pneumonia invasion into bighorn sheep populations. Healthy years also occurred periodically, and understanding the factors driving these apparent fade-out events may be the key to managing this disease. Our data and modelling indicate that pneumonia can have greater impacts on bighorn sheep populations than previously reported, and we present hypotheses about processes involved for testing in future investigations and management.

Key-words: bacterial pneumonia, livestock-wildlife interface, Markov model, time series

Introduction

Over the past 20 years, considerable advances have been made in understanding the spatio-temporal patterns of disease persistence and fade-out following invasion into susceptible host populations. Infections that generate

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rapid mortality such as Ebola virus, burn through susceptible populations until there are no more hosts and effectively die out (Sanchez *et al.* 2001). Infections with a strong immunizing effect, such as measles in England and Wales, persist in populations and exhibit biannual epidemic peaks that coincide with the birth and aggregation of sufficient susceptibles (Bjørnstad & Grenfell 2008). The dynamics of strong immunizing or fatal infections can leave a distinct spatio-temporal signature, although an infection that results in predictable disease in one instance, may appear almost chaotic in another setting; for example, contrast the dynamics of measles in the UK and Niger (Ferrari *et al.* 2008). Describing these spatio-temporal patterns can reveal underlying processes and this approach can be especially important in understanding infections that have recently invaded a population where the transmission routes or aetiological agents are not clear (Cleaveland *et al.* 2007). In this article, we examine the spatio-temporal dynamics of pneumonia in bighorn sheep, where the disease has been described for at least 80 years (Rush 1927), but debate continues about the identities and roles of causal agents, and disease remains an important factor limiting recovery of populations.

Bighorn sheep (*Ovis canadensis*) are social, sexually dimorphic ungulates. The species commonly occurs in spatially structured, demographically independent, interconnected populations in steep, rugged terrain. Males and females pursue different life-history strategies (Bleich *et al.* 1996; Rubin, Boyce & Caswell-Chen 2002). Interactions between the sexes are concentrated around the breeding season which is relatively short in northern latitudes and high altitudes (Bunnell 1982; Thompson & Turner 1982; Bleich, Bowyer & Wehausen 1997; Valdez & Krausman 1999). Seasonal breeding also governs contact patterns between age classes, and each year a pulse of neonates is reared in female-juvenile nursery groups. Outside the breeding season, mature males and females generally occur in male-only, female-only or female-offspring associations. Males are more mobile and more likely than females to contact conspecific hosts in adjacent populations, or potential disease reservoirs such as domestic sheep (Bleich, Bowyer & Wehausen 1997; Rubin *et al.* 1998; DeCesare & Pletscher 2006).

Pneumonia is a significant factor limiting the distribution and abundance of bighorn sheep (Gross, Singer & Moses 2000; Cassirer & Sinclair 2007; Boyce *et al.* 2011). The disease is associated with infection by directly transmitted bacteria, principally thought to be *Mycoplasma ovipneumoniae* and *Mannheimia haemolytica*, but, as is often the case with pneumonia, the precise aetiology remains unclear (Foreyt, Snipes & Kasten 1994; Besser *et al.* 2008, 2012b; Dassanayake *et al.* 2009, 2010). Initially, infection probably originates in domestic sheep, but once it has spilled over into bighorn sheep populations it is most likely maintained in the population and spread by bighorn sheep. Bighorn sheep appear highly susceptible to infection from domestic sheep: nearly all (98%) of a total of 90 bighorn sheep that were co-pastured with domestic

sheep in 11 experimental commingling studies conducted between 1979 and 2009 died of pneumonia within 100 days, while the domestic sheep remained healthy (summarized in Besser *et al.* (2012a). Although these captive experimental results support field observations by naturalists and field biologists (Grinnell 1928; Shillenger 1937; Goodson 1982; George *et al.* 2008), they do not replicate the range of demographic variation in pneumonia events observed under natural conditions. Pneumonia described in free-ranging bighorn sheep populations includes acute die-offs with wide ranges in all-age mortality (10–90%), chronic or sporadic low levels of adult mortality, and annual or sporadic epizootics with high mortality rates restricted to juveniles from 1 to many (>20) years following all-age outbreaks (Rush 1927; Jorgenson *et al.* 1997; Aune *et al.* 1998; Enk, Picton & Williams 2001; Hnilicka *et al.* 2002). The aim of this paper was to use empirical data to describe these mortality patterns in detail and to develop hypotheses about the underlying processes involved. Indeed, a lack of data has so far constrained models of pneumonia dynamics in bighorn sheep (Hobbs & Miller 1992; Gross, Singer & Moses 2000; Clifford *et al.* 2009; Cahn *et al.* 2011). Our objective was to develop an understanding of the disease that will ultimately aid in identifying and assessing intervention options.

Materials and methods

STUDY AREA

We studied bighorn sheep in a 22 732 km² area encompassing Hells Canyon of the Snake River in the Blue Mountain and Columbia Plateau ecoregions of Idaho, Oregon and Washington (–117.875°, 46.500° to –116.250°, 44.750°, Fig. 1). Bighorn sheep occupy three climate zones within this diverse area from lowest to highest elevation: Snake River, Blue Mountains and Wallowa Mountains. The low elevation Snake River canyon is warm and dry with temperatures averaging 17.6 °C at Lewiston, ID. Average annual precipitation of 31.4 cm occurs fairly evenly year-round except during the months of July and August. The adjacent uplands including the Blue Mountains in Washington, are cooler and wetter with average temperatures of 10 °C in Pomeroy, Washington (WA) and average annual precipitation of 61 cm at Asotin, WA and 66 cm in Pomeroy. The upper elevations in the Wallowa and Seven Devils mountains receive annual precipitation of up to 205 cm, over two-thirds of which occurs as snow. Temperature averages 7 °C at the base of the Wallowa Mountains in Enterprise, OR and annual precipitation averages 76 cm. Seasonal temperature patterns in all three climate zones are similar, with highs in July and August and lows in December and January (Johnson & Simon 1987; Western 2008).

Bighorn sheep are native to Hells Canyon, but were extirpated by 1945, probably through a combination of unregulated hunting, competition with livestock for forage and diseases introduced from domestic sheep (Smith 1954; Johnson 1980; Coggins & Matthews 1996). From 1971 to 1995, wildlife agencies in Idaho, Oregon and Washington translocated a total

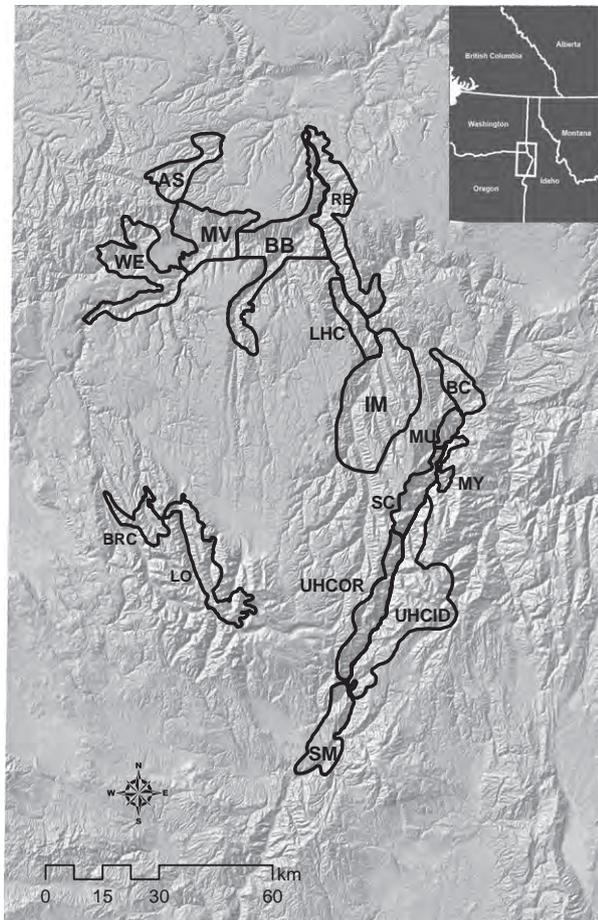


Fig. 1. Distribution of the 16 bighorn sheep populations in the Hells Canyon metapopulation, Idaho, Oregon and Washington. AS = Asotin; WE = Wenaha; BB = Black Butte; BC = Big Canyon; BRC = Bear Creek; IM = Imnaha; LHC = Lower Hells Canyon; LO = Lostine; MU = Muir; MV = Mountain View; MY = Myers Creek; RB = Redbird; SC = Upper Saddle Creek; SM = Sheep Mountain; UHCID = Upper Hells Canyon, Idaho; UHCOR = Upper Hells Canyon, Oregon.

of 329 bighorn sheep into Hells Canyon and moved 79 within the metapopulation, establishing 12 interconnected populations prior to our study (Figure S1). Another four populations were established and one population supplemented with translocations 1997–2005, during our study. Populations were delineated by movement patterns of females (Rubin *et al.* 1998). Females rarely move between populations whereas males may move seasonally or disperse among populations. Periodic pneumonia outbreaks were documented prior to this study, although monitoring was sporadic and most pneumonia events were documented following reports of sick and dying sheep. Over the same time period, domestic sheep grazing declined dramatically. However, reduced numbers of domestic sheep and goats continue to graze intermittently on public and private lands. Active management is ongoing to prevent contact between species: 22 bighorn sheep, five domestic goats and three domestic sheep were removed from areas where there was risk of contact during the study, nonetheless, some potential for disease transmission from domestic sheep and goats existed for all bighorn sheep populations throughout the study.

MONITORING

In 1995 and 1996, all-age pneumonia outbreaks occurred in five populations in the northern part of the project area (Cassirer *et al.* 1996). In 1997, we started monitoring movements and survival of radio-collared bighorn sheep in three of these populations (Redbird, Black Butte and Wenaha) as part of an unsuccessful vaccination trial to improve lamb survival (Cassirer *et al.* 2001). We collared animals in additional populations in 1998, 1999, 2000, 2006 and 2010 including animals that were translocated and, as animals left the study due to death or were censored due to radio failure, we replaced them by collaring new individuals.

State wildlife agencies have conducted periodic ground and aerial surveys since initial reintroductions in 1971. Between 1997 and 2010, annual helicopter surveys were conducted between February and April. Visibility of sheep is high (87%), as determined by detection of radio-collared animals (Idaho Fish and Game data) and population estimates were derived by combining helicopter counts with observations from ground and observations from fixed-wing monitoring of radio-collared animals. Most lambs were born in May and we conducted our population analyses on a biological year, May–April. Annual exponential rate of population increase was calculated as $r = \ln(N_t/N_{t-1})$. During this period, 735–900 bighorn sheep were estimated to occur within the metapopulation. Estimated population sizes ranged from 5 to 190, with a median of 35.

We calculated annual adult survival by sex as the proportion alive in May that survived to the following May in populations with at least five radio-collared animals. Summer lamb survival was the proportion of known offspring of radio-collared ewes that survived until October (approximately to weaning). We classified a female as having a lamb when she was observed alone with, or nursing a lamb. We assumed lambs were dead when the female was no longer associating with a lamb. We located dead lambs through visual observation. We defined recruitment as the ratio of lambs to ewes recorded in the annual February–April surveys.

We located radio-collared sheep at least bi-weekly from the ground or from fixed-wing aircraft. We located females up to several times per week during lamb-rearing to monitor productivity and lamb survival. Radiocollars were equipped with a motion-sensitive switch. When no movement was detected for 4 h, the switch was activated and we conducted an investigation on site and collected the entire carcass or tissue samples for analysis at the Washington Animal Disease and Diagnostic Laboratory (WADDL), Washington State University, Pullman. On the basis of site investigations and necropsy results, we classified causes of death as disease, predation, accident or injury, human-caused or unknown. We censored animals that died within 30 days of capture and animals translocated to Hells Canyon did not enter the study until the start of the biological year following translocation (2–4 months following release).

PATHOLOGY

We based diagnoses of pneumonia on gross and histological examination of lung tissue at WADDL. Gross features used to diagnose pneumonia included consolidation, presence of lung adhesions, abscesses, bronchiectasis or pleuritis. Affected areas of the lung were characterized by tissue colour, consistency and ability to float in formalin. Histological features of acute pneumonia included fibrin and oedema, increased presence of pulmonary macrophages, neutrophils, necrotic neutrophils,

necrosis, haemorrhage and bacterial colonies in lung tissue. Chronic pneumonia was characterized by the presence of fibrosis, abscesses or bronchiectasis. Bronchiolar epithelial hyperplasia and peribronchiolar lymphocytic infiltrates in the absence of fibrosis or abscessation was designated as subacute pneumonia. Severity (mild, moderate or severe) was based primarily on the percentage of both right and left lung fields affected on gross examination. Severity assessed by histopathology was based on the total percentage of affected tissue on individual sections of lung. Five to 15% total affected lung or tissue was considered mild, > 15–50% was moderate and > 50% was severe.

HEALTH STATUS

We used confirmed and suspected (for lambs) pneumonia-caused mortalities to characterize the seasonality, duration and intensity of four types of pneumonia events by population and year: (i) all-age pneumonia, (ii) secondary all-age pneumonia, (iii) adults only, and (iv) lambs only. We classified a population-year as healthy if animals were radiocollared in the population, but we did not detect any pneumonia in adults or detect or suspect pneumonia in lambs as described in the results.

ANALYSIS

We used Mann–Whitney's U test and Wilcoxon's Rank Sum (Siegel & Castellan 1988) to compare median survival rates of adults and juveniles and population growth by health class due to lack of normality in the data (Shapiro-Wilks test $P < 0.0001$). We analysed seasonal patterns in lamb survival to weaning by translocation status and climate zone with Kaplan–Meier estimates and log-rank tests (Kaplan & Meier 1958).

We fit Bayesian survival models to analyse the effect of pneumonia on the daily mortality risk from birth to 140 days in lambs. Starting at day 0 (birth), we used a piecewise-constant hazard approach where the instantaneous daily mortality hazard, $h(a)$, was assumed to be constant for each day. Daily hazard estimates were smoothed using a first order conditional autoregressive approach, $h(a) = \exp(\beta + y(a))$, where β is a global intercept with an improper flat prior distribution and $y(a)$ was specified using the `car.normal` function in WinBUGS assuming a $Uniform(0,10)$ hyperprior on σ , and τ , the `car.normal` precision parameter, set equal to $\frac{1}{\sigma^2}$ (Besag, York & Mollie 1991; Heisey *et al.* 2010). We used Markov chain Monte Carlo methods to generate separate posterior distributions for daily mortality hazards by health class (pneumonia or healthy). We ran three Markov chains for 100 000 iterations, discarded the first 50 000 steps, and thinned the remaining steps so that our posterior included every 10th iteration. The Markov chains readily converged (Gelman-Rubin statistic ≤ 1.13 for healthy years, and ≤ 1.02 for pneumonia-years). Further details are provided in Appendix S1.

To identify significant seasonal clustering in adult pneumonia mortalities, we fit a logistic regression model to a series of seasons. The response was a binomial equal to the proportion of adult pneumonia mortalities occurring in that season weighted by month, and the predictor was a binary season indicator for 'summer' or 'winter'. We varied the months categorized as summer by starting with the lamb-rearing months, May–August, and classifying all other months as 'winter' and systematically extended the endpoints of the summer season. We present the grouping that showed the greatest difference between seasons.

In populations where we documented pneumonia during the study (we excluded the healthy Asotin and Upper Saddle Creek populations), we used health status in the current year (a categorical predictor taking on separate values for all-age pneumonia, adult-only pneumonia, lamb-only pneumonia or healthy, with healthy as the baseline) as a predictor for future pneumonia (coded as 0 if the next year was healthy, and 1 otherwise). To test for differences among translocated and resident populations, logistic regression models were of the form, $\frac{\pi(x)}{1-\pi(x)} = e^{\alpha_i + \beta_i X_i}$ where e^{α_i} is the odds of pneumonia this year given last year's health status and $e^{\beta_i X_i}$ is the multiplicative adjustment to these odds accounting for the population's translocation status, X_i (an indicator taking on the value 0 for resident populations and 1 for translocated populations). We used Firth's bias-reduction technique for complete separation (Firth 1993) because we always observed pneumonia the year following all-age pneumonia.

We estimated annual transition probabilities between pneumonia classes for populations that had experienced epizootics by building a matrix from the frequency of transitions between classes during the study. Since the transition matrix was regular and irreducible (any state could potentially transition to any other state), we derived the stationary distribution by repeatedly multiplying the probability transition matrix by itself until row values converged (*c.* 15 iterations) (Taylor & Karlin 1998).

To assess the evidence for spatial synchrony of pneumonia, we used logistic regression to evaluate the influence of pneumonia status in neighbouring populations on a population's odds of pneumonia. We calculated centroids of 95% contours of fixed kernel home ranges of radiolocations of resident animals by population in Hawth's Tools (Beyer 2004) and ArcMap 9.3 (ESRI 2008). We defined a population's neighbours to be all populations with centroids within a designated Euclidean distance (from 10 to 70 km) of the population of interest. Pneumonia in neighbours was a categorical predictor that took on the value 1 if any neighbouring population had pneumonia in the year of interest, and 0 otherwise. We included years when pneumonia was known to be present in the neighbourhood, even if some neighbours were not sampled. We recognize that our probability of detecting pneumonia was less than 1, so we excluded data points (range from 26 to 53% of points at each distance category) where no pneumonia was detected in neighbours, but not all neighbours were sampled. Since a population's pneumonia status in year $t-1$ altered its pneumonia odds in year t , we included last year's pneumonia status in both the population of interest and the neighbouring populations as predictors in the models. To evaluate the effect of translocations, we added an indicator variable for translocated populations in the neighbourhood.

Data were analysed in the R statistical computing environment (R Development Core Team, 2008) through the `lme4` (Bates, Maechler & Dai 2008) and `logistf` (Pioner *et al.* 2006) packages. The lamb mortality hazard model was fit in WinBUGS version 1.4 (Lunn *et al.* 2000) through R version 2.13.0 using the `R2WinBUGS` package (Sturtz, Ligges & Gelman 2005).

Results

PNEUMONIA IN ADULTS

Between 1997 and 2010, 477 bighorn sheep were radiocollared (313F, 164M) in 14 populations (Fig. 1) and monitored for a total of 141 population-years (1–14 years

per population). On average, 117 radio-collared adults (range 35–146) were monitored each year, with a median of 24% (range 5–100%) of adults collared in each study population (Table S1). This included 339 resident sheep monitored for 1220 sheep-years. Another 104 sheep translocated to Hells Canyon from presumably healthy populations in British Columbia, Alberta and Montana 1997–2002, and 34 sheep that were moved within the Hells Canyon metapopulation 1999–2005 were monitored for a total of 459 sheep-years. The translocations established the Big Canyon, Muir Creek, and Myers Creek and Saddle Creek populations, and supplemented existing populations at Asotin, Upper Hells Canyon Oregon, Lostine and Bear Creek (Table S1 and Figure S1).

We determined a cause of death for 179 of 264 radio-collared bighorn sheep (94M, 170F) that died and 53 (30%) were diagnosed with bacterial pneumonia (17M, 36F). We also found 12 (8M, 4F) unmarked dead adult sheep that were diagnosed with bacterial pneumonia. Pneumonia-caused mortality of radio-collared sheep was 27% (28 of 104) of translocated animals and 7% of radio-collared resident animals (25 of 339, $\chi^2 = 28.87$, 1 d.f., $P < 0.01$).

PNEUMONIA IN LAMBS

We submitted 129 unmarked dead lambs from 14 populations for necropsy and euthanized 11 live lambs in four populations. We determined a cause of death for 104 lambs and 92 (88%) were diagnosed with pneumonia including 9 of 11 euthanized lambs (Besser *et al.* 2008). Although juveniles of all ages died from pneumonia, most mortality was prior to weaning, between 4 and 14 weeks of age (Fig. 2). We found no differences in the summer survival distribution functions of lambs in years with pneumonia among the Snake River, Blue Mountains and Wallowa Mountains climate zones ($\chi^2 = 0.1$, 2 d.f.,

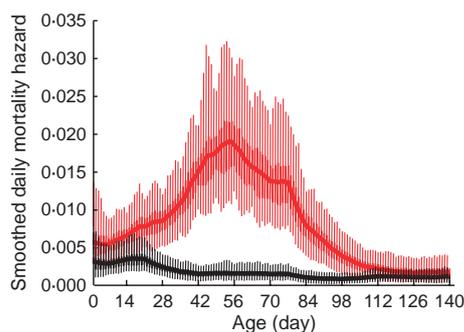


Fig. 2. Daily mortality hazard from 0 to 140 days of lambs born to radio-collared ewes in population-years where no pneumonia was documented (in black, 267 lambs) and where pneumonia was diagnosed (in red, 262 lambs). Solid line is the smoothed daily hazard, dark and light bars represent 50% and 95% credible intervals from a conditional autoregressive model. The 95% credible intervals for lamb hazards in pneumonia and healthy population-years did not overlap between the ages of 27 and 101 days.

$P = 0.97$) or between lambs of translocated and resident ewes ($\chi^2 = 1.5$, 1 d.f., $P = 0.23$).

Due to the difficulty of detecting freshly dead unmarked lambs in a large, relatively inaccessible and rugged landscape, we assigned a class of ‘suspected pneumonia’ in lambs based on (1) the distinct temporal signature of documented pneumonia-induced mortality in 37 lamb-only or secondary all-age population-years (Fig. 2); and (2) observations of clinical signs of pneumonia including lethargy, coughing, nasal discharge and discovery of intact dead lambs that were too autolysed for diagnosis. We were conservative in assigning the suspected class of pneumonia to lambs. Median summer lamb survival and recruitment (lamb : ewe ratio) were higher or did not differ in population-years with documented vs. suspected pneumonia (Fig. 3).

HISTOPATHOLOGY

Lung lesions observed at necropsy included acute fibrinous bronchopneumonia and pleuritis, sub-acute broncho-interstitial pneumonia with lymphocytic cuffing of airways and bronchiolar hyperplasia, and chronic pneumonia with fibrosis and abscessation. Acute lesions were observed in approximately half of the mortalities regardless of age class (30 of 65 adults and 33 of 66 lambs). Chronic lesions were present in about half (33) of the adult mortalities compared with about a quarter of the lambs (15). Sub-acute lesions were more common in lambs ($n = 18$, 27%) than in adults ($n = 2$, 3%).

SEASONAL PATTERNS

There was no difference between sexes in monthly patterns of pneumonia-caused adult mortality ($\chi^2 = 6.77$, d.f. = 11, $P = 0.82$). In both sexes, the odds of pneumonia-caused mortalities were almost three times higher between October and February than during the rest of the year (odds ratio 2.85, 95% CI 1.7–4.8, $P < 0.0001$). The seasonal pattern was driven by mortalities with acute lesions (odds ratio 4.29, 95% CI 1.7–10.9, $P = 0.002$). Deaths of animals with chronic lesions were more evenly distributed across seasons (odds ratio 1.9, 95% CI 1.0–4.0, $P = 0.05$). No acute pneumonia was detected in adults between May and July, the period when most (80%) pneumonia mortalities were detected in lambs. Peak pneumonia mortalities in lambs at 1–3 months of age corresponded to the period when ewes congregated in nursery groups and mortalities associated with pneumonia in adults peaked during the breeding season when mixed sex group sizes were largest (Fig. 4).

TEMPORAL AND SPATIAL PATTERNS

Pneumonia was detected or suspected in 33–77% of the study populations each year. Two populations remained healthy throughout the study: Asotin and Saddle

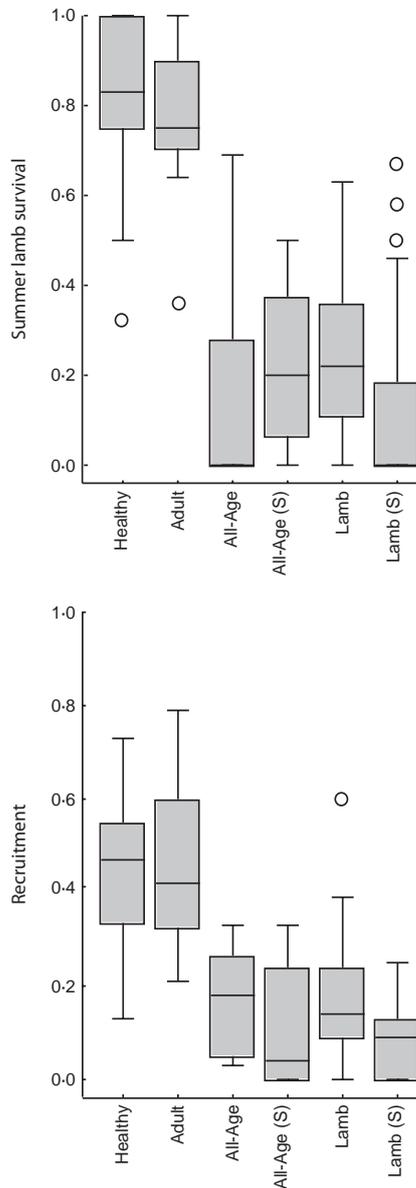


Fig. 3. Summer lamb survival and recruitment in healthy, adult-only, all-age and lamb pneumonia-years vs. suspected all-age or lamb pneumonia-years. The horizontal line denotes the median, the box encloses 50% of the observations and the whiskers show the 2.5th and 97.5th percentiles. Median summer lamb survival and recruitment did not differ significantly between all-age pneumonia population-years when pneumonia was detected in both adults and lambs and population-years when pneumonia was detected in adults and suspected in lambs ($W \leq 48$, $P > 0.10$).

Creek (Fig. 5). Survival and population growth patterns differed significantly among age-structured health classes, indicating that pneumonia was a dominant and additive source of mortality (Table 1).

Pneumonia restricted to lambs (lamb-only) was the most frequent class of pneumonia observed, and populations usually remained stable (Table 1). Pneumonia in both adults and lambs simultaneously (all-age) occurred in translocated populations in biological years 2000, 2002

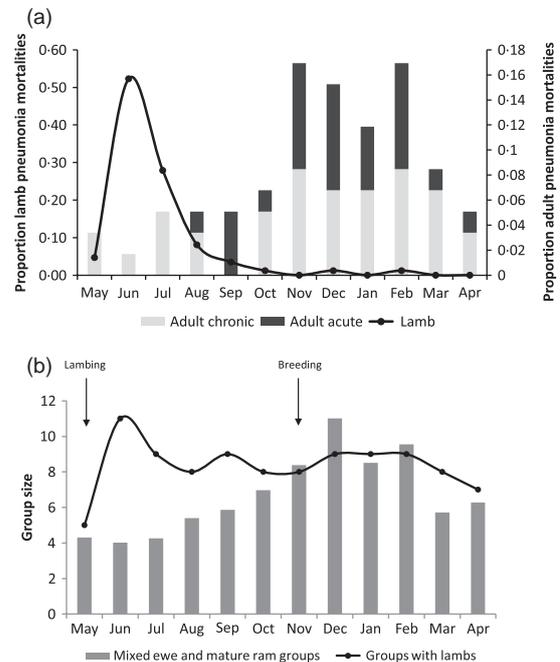


Fig. 4. Seasonal patterns of pneumonia and life-history events. (a) Monthly distribution of pneumonia mortalities detected in adults and lambs. (b) Median group sizes of groups with lambs and ewe-mature ram groups by month.

and 2003. This accounted for 68% (19 of 28) of the pneumonia mortalities in translocated animals and resulted in immediate population declines. Secondary all-age pneumonia events occurred in both resident and translocated sheep in populations that had previously experienced all-age outbreaks. These events were characterized by summer pneumonia outbreaks in lambs followed by lower rates of pneumonia-induced mortality in adults. Pneumonia in adults only was an infrequent, usually low mortality event (Table 1).

We observed high survival and stable to increasing populations in population-years classified as healthy, even in populations with a previous history of pneumonia. However, once pneumonia invaded a population, healthy periods were usually of short duration (median 1 year, range 1–3 years, Table 1, Fig. 5).

Median Euclidian distance between population centroids was 67 km with a range from 1 (populations separated by the Snake River) to 156 km (Fig. 5). We detected no significant differences in probability of pneumonia relative to distance to neighbouring populations with pneumonia. There was a slight, but insignificant increase in probability of adult or all-age pneumonia-years in populations centred 20 km or less apart ($\beta_{\text{NeighbourPN}} = 0.97$, $SE = 0.76$, $P = 0.20$) and no spatial correlation of pneumonia in lambs (Figure S2). Adding a 1-year lag or an indicator for the presence of translocated populations in the neighbourhood did not alter this result ($P > 0.32$).

We found a significant predictive effect of current pneumonia class on health status of the population the follow-

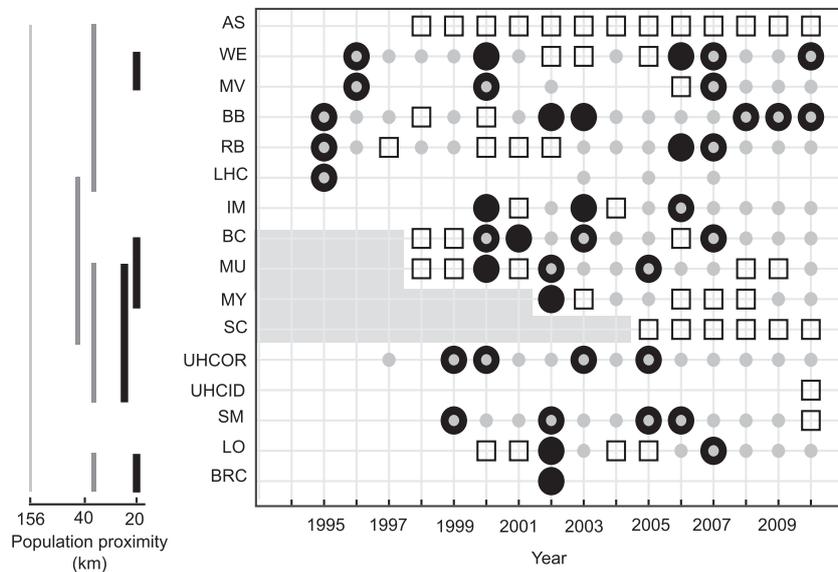


Fig. 5. Fourteen year time series of pneumonia classes in 16 populations in the Hells Canyon bighorn sheep metapopulation 1995–2010. Black circles represent documented pneumonia in adults, small grey dots represent documented or suspected pneumonia in lambs, open squares indicate no pneumonia detected or suspected. White background with no symbols indicates no data. Grey background indicates years prior to establishment of population through translocation. Vertical lines to the left of the plot connect population centroids at three distance scales. All population centroids were within 156 km or less. AS = Asotin; WE = Wenaha; MV = Mountain View; BB = Black Butte; RB = Redbird; LHC = Lower Hells Canyon, Oregon; IM = Imnaha; BC = Big Canyon; MU = Muir; MY = Myers Creek; SC = Upper Saddle Creek; UHCOR = Upper Hells Canyon, Oregon; UHCID = Upper Hells Canyon, Idaho; SM = Sheep Mountain; LO = Lostine; BRC = Bear Creek.

ing year. Continued pneumonia, usually in lambs, was most likely following all-age and secondary all-age (98%) or lamb-only pneumonia-years (83%). The probability of a pneumonia-year following adult-only and healthy years was similar (63% and 62%, respectively, $P = 0.98$), and pneumonia was significantly less likely after healthy years than all-age or lamb pneumonia-years ($P \leq 0.05$, Table 2).

We used the observed frequency of transitions between health classes to develop a transition matrix (Table 2) with Markov properties: there were a finite number of health classes (or states), health class in the current year was dependent on health in the previous year, and any health class could transition to any other health class. Thus, we could predict the stationary distribution of health classes. Assuming transition probabilities among health classes remain constant, pneumonia is predicted in 81% of populations annually: lamb-only pneumonia 57%, all-age and secondary all-age pneumonia combined 17%, adult-only pneumonia 7%. To further illustrate the dynamics of pneumonia-induced mortality, we combined the stationary distribution with mortality and transition rates (Tables 1 and 2) for a visual representation of the impact of disease over time (Fig 6).

Discussion

Analysis of a 14-year time series of pneumonia in 16 interconnected bighorn sheep populations revealed that

age-structured classes of pneumonia and healthy years had markedly different demographic impacts on populations. All-age pneumonia was consistently associated with population declines, but ultimately, lambs carried the greatest burden of disease. Rates of pneumonia-induced mortality in lambs can vary significantly by population and year, but on average, pneumonia in lambs had an even greater impact than previously reported (Clifford *et al.* 2009; Cahn *et al.* 2011). Recurring annual pneumonia epizootics in lambs may pose the greatest threat to population recovery, and when accompanied by high adult survival, the true consequences of disease may not be realized until senescent adults die and are not replaced.

While pathogen invasion, reinvasion, persistence and fade-out can't be confirmed in the absence of known disease agents, we can evaluate evidence for these processes to develop hypotheses for future investigation. High initial all-age mortality, when compared with subsequent adult mortality in translocated and resident populations is consistent with invasion of pathogens into groups of apparently naïve individuals. Pneumonia in lambs after all-age events must be due to infection from carrier ewes as lambs have little contact with other potential sources of pathogens prior to weaning (Festa-Bianchet 1991; Bleich, Bowyer & Wehausen 1997). Lamb pneumonia outbreaks have also been described in captivity with similar conclusions (Foreyt 1990; Ward *et al.* 1992; Cassirer *et al.* 2001). Pneumonia in lambs is thus a good indication of

Table 1. Demographic characteristics of health classes in 14 Hells Canyon bighorn sheep populations, 1997–2010. Data reported as median (range). Years where no adults were radiocollared were excluded from analyses

Outbreak class	<i>n</i>	<i>n</i> pneumonia cases confirmed	Consecutive years	Population growth (<i>r</i>)	Ewe survival ^a	Ram survival ^a	Summer lamb survival	Spring lamb:ewe ratio
All-age	3	28 Adult	1 (1, 1)	-0.34 (-0.42, -0.28)	0.50 (0.29, 0.57)	NA	0.71 (0.29, 0.88)	0.13 (0.06, 0.17)
Secondary all-age	16	(20 Adult, 27 Lamb)	1 (1, 1)	0 (-0.17, 0.03)	0.82 (0.60, 1)	0.75 (0.67, 1)	0.10 ^b (0, 0.69)	0.05 ^b
Adult only	11	14	1 (1, 2)	0.03 (-0.17, 0.16)	0.83 (0.67, 1)	0.74 (0.67, 0.83)	0.75 (0.36, 1)	0.42 (0.21, 0.79)
Lamb only	62	65	2 (1, 4)	0 (-0.69, 0.22)	0.93 (0.56, 1)	0.83 (0.60, 1)	0.20 ^b (0, 0.67)	0.14 ^b (0, 0.60)
Healthy	49	0	1 (1, 13)	0.12 (-0.03, 0.37)	0.93 (0.71, 1)	0.90 (0.71, 1)	0.83 (0.33, 1)	0.47 (0.13, 0.73)

^aSurvival of radio-collared ewes and rams in years where *n* = 5 or more.

^bSurvival in years with documented pneumonia in lambs (*n* = 8 secondary all-age, *n* = 33 lamb-only).

infection and pathogen shedding in ewes. The absence of pneumonia-induced mortality or clear symptoms in these ewes during outbreaks in lambs confirms that they have either developed resistance or perhaps tolerance of the pathogen(s) that are lethal to their offspring (Råberg, Graham & Read 2009). Reasons for more frequent fade-out following years with pneumonia restricted to adults remains unclear, but could be explained by differences in pathogens, host immunity or transmission rates.

Our study confirms previously reported accounts of seasonality of pneumonia deaths in bighorn sheep, a pattern commonly observed in infectious diseases of humans and wildlife (Spraker *et al.* 1984; Aune *et al.* 1998; Enk, Picton & Williams 2001; Altizer *et al.* 2006; Cassirer & Sinclair 2007). Age-specific seasonal patterns in pneumonia mortality corresponded to breeding and lamb-rearing: life-history events that are accompanied by especially intensive and concentrated social interactions. The distinct seasonality of adult pneumonia mortality observed in wild populations is not observed in captive experimental bighorn and domestic sheep commingling trials where bighorn sheep die of pneumonia regardless of season. Seasonal physiological or environmental factors are therefore probably less important in precipitating pneumonia epizootics than the timing of pathogen introduction, pathogen virulence and exposure to infections (contact rates). The lack of synchrony of disease events across populations and the absence of an effect of climate on lamb survival during pneumonia-years also suggest that weather or other landscape-scale extrinsic variables (Grenfell *et al.* 1998; Cattadori, Haydon & Hudson 2005), are unlikely to be important drivers of pneumonia in Hells Canyon.

In lambs, most pneumonia-induced mortality occurred between 1 and 3 months of age, a period that coincided with aggregation in nursery groups. Lamb-to-lamb contact may be an important route of infection as happens in many directly transmitted human 'childhood diseases'; thus, the synchrony in parturition and subsequent concentration of ewes during lamb-rearing which is typical in northern latitudes, could contribute to the timing and high rates of mortality. This period also coincides with the age when passively acquired immunity is probably waning in lambs (Rajala & Castrén 1995), which would further promote transmission and mortality.

By analysing long-term monitoring data to elucidate disease processes from patterns of mortality, we have diverged from studies of bighorn sheep pneumonia that focus on identifying the primary causal agent. The benefits of such a study were that we were able to examine demographic patterns at comparatively large spatial and temporal scales, allowing us to make inferences about processes such as disease introduction, persistence and fade-out. However, the weakness in our approach is an inability to track a known pathogen and directly measure transmission (i.e. infection may occur long before mortality); no opportunity to verify pathogen absence during healthy years;

Table 2. Temporal pattern of pneumonia within affected populations: annual probabilities of transition among health states and annual probability of any pneumonia. Populations that remained healthy throughout the study, population-years before the initial observation of pneumonia, and years where no adults were radiocollared were excluded from analyses (Fig. 5 and Table S2)

Initial state	n	Transition state				Probability of any pneumonia following initial state (95% CI; P-value relative to healthy state)
		All-age ^a	Healthy	Adult	Lamb	
Healthy	24	0.13	0.33	0.08	0.46	0.62 (0.4, 0.8)
All-age ^a	17	0.18	0.00	0.06	0.72	0.97 (0.8, 1; $P < 0.01$)
Adult	11	0.18	0.36	0.09	0.36	0.63 (0.3, 0.9; $P = 0.98$)
Lamb	54	0.15	0.19	0.07	0.59	0.82 (0.6, 0.9; $P = 0.05$)

^aAll-age and secondary all-age classes combined.

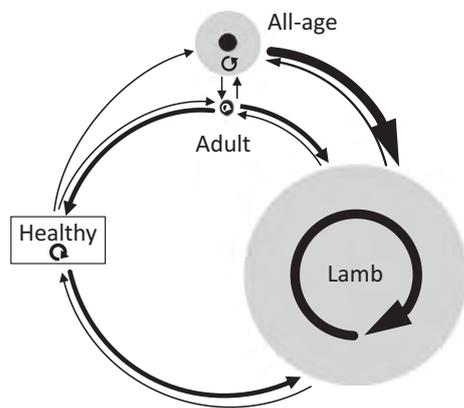


Fig. 6. Long-term patterns of pneumonia mortality in bighorn sheep populations experiencing epizootics, Hells Canyon 1997–2010. Stationary distribution of four age-structured population health classes (all-age includes secondary all-age) and the probabilities of staying within a class or transitioning out. Pneumonia classes are circles scaled by relative frequency multiplied by median death rates of ewes (black) and/or lambs (grey) in the class. The healthy class is not scaled. The thickness of arrows between classes is proportional to transition probabilities (Table 2).

and no possibility to monitor genetic variation in the pathogen over time. Given these limitations, as well as the usual constraints of marking and monitoring animals in the field, a primary concern is an imperfect detection probability for pneumonia, which could lead to overestimating healthy population-years. However, the likelihood of detecting pneumonia was not correlated with the intensity of monitoring as measured by the proportion of the population that was radiocollared (median in suspected and detected pneumonia-years = 0.22; in healthy years = 0.28, $U = 3331.5$, 1 d.f., $P = 0.09$, Tables S1 and S2), or the frequency of locations (median locations per animal per year in suspected and detected pneumonia-years = 30; in healthy years = 32; $U = 2427.5$, 1 d.f., $P = 0.45$). Therefore, there was no bias towards monitoring populations with pneumonia and, despite potentially misclassifying some lower mortality pneumonia events, we still detected significant differences in population dynamics between several different classes of pneumonia and healthy years. Survival and population growth were also similar in years

classified as healthy in populations with and without a history of pneumonia, suggesting that healthy years, with true absence of disease-related mortality (but not necessarily true absence of infection), did occur, even in populations with previous pneumonia, and these classifications are useful and appropriate for describing the system.

Our observations concur with many of the results of previous studies, but also raise questions about disease models that assume all-age pneumonia outbreaks followed by lamb mortality at a constant or declining rate for a period of usually 1–6 years (Gross, Singer & Moses 2000; Clifford *et al.* 2009; Cahn *et al.* 2011). We observed that pneumonia persisted within populations (or was periodically reintroduced) consistently longer than previous models have assumed, and, as indicated by the Markov model stationary frequency distribution, continued to affect all-age classes, not just lambs. The consequence is that all-age pneumonia events can result in sporadic or chronic, long-term reduction of survival of both adult and juvenile age classes. The disparity between our findings and previous studies may be due to the greater sampling intensity, duration and spatial scale of our study. Furthermore, whereas initial invasion associated with high rates of mortality is fairly easy to detect, the end of an epizootic is not always clear. Previously published models assume that low mortality or healthy years represent the pathogen extinction and the end of the epizootic. However, if disease in a long-lived animal like bighorn sheep is accompanied by latent periods and low rates of mortality in chronically infected animals, absence of mortality may not reflect absence of pathogens. Long-term dynamics could be a function of changes in immune status in individuals and include stochastic events common to small populations, such as dispersal, colonization, recruitment, death, intermittent pathogen shedding or lambing status of asymptomatic carriers.

By analysing long-term patterns, we have generated hypotheses about the disease processes associated with pneumonia epizootics in bighorn sheep. As with other diseases with high levels of heterogeneity, these processes are probably affected by a number of factors, including previous exposure of hosts, pathogen dose or virulence, and spatial structuring and contact rates in host popula-

tions (Grassly & Fraser 2008; Salkeld *et al.* 2010; Wendland *et al.* 2010; Jesse & Heesterbeek 2011). On the basis of the patterns we observed, the disease appears to be an infection that, in some ways is similar to measles and other immunizing diseases in humans in that it spreads through all-age classes during invasion, but subsequently mainly affects susceptible juveniles. However, in contrast with measles, pathogens apparently persist, occasionally causing fatal pneumonia in previously exposed adults, and the variable lung lesions and associated bacteriology suggest a polymicrobial aetiology, thus secondary pathogens may play a role in severity and recurrence (Besser *et al.* 2012b). The course of the disease may also be affected by the timing of pathogen invasions relative to contact rates associated with seasonal breeding and parturition. The importance of between-population transmission and recurrent infection from domestic sheep deserves additional investigation as do the conditions that lead to disease and pathogen fade-out.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Lamb mortality hazard analysis.

Figure S1. Demographic histories of Hells Canyon bighorn sheep populations.

Figure S2. Spatial synchrony of pneumonia-years across populations.

Table S1. Number and proportion of adult bighorn sheep radio-collared in each population by year and translocation status.

Table S2. Health classification of population-years (141) used to estimate demographic characteristics and transition probabilities of pneumonia and healthy years in Hells Canyon, 1997–2010.

APPENDIX II
PROGRESS REPORTS FOR STUDIES IN STATEWIDE BIRD ECOLOGY

STUDY 1: GREATER SAGE-GROUSE (*Centrocercus urophasianus*) HABITAT AND POPULATION TRENDS IN SOUTHERN IDAHO

Job I: Factors Influencing the Ecology of Greater Sage-Grouse Inhabiting the Bear Lake Plateau and Valley, Idaho-Utah

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ABSTRACT: The purpose of this research is to describe the ecology, seasonal movements, and habitat-use patterns of greater sage-grouse (*Centrocercus urophasianus*) that inhabit the Bear Lake Plateau and Valley relative to existing land-uses. Because the Bear Lake Plateau and Valley is subject to both natural and anthropogenic barriers and fragmentation, defining population vital rates, seasonal movements, and habitat-use relative to land use and jurisdictional boundaries of this population will provide an important foundation for management cooperation among Idaho, Utah, and Wyoming. Preliminary data are provided. Sage-grouse land use research will also define the core use areas of important seasonal and temporal habitats in the Bear Lake Plateau and Valley.

INTRODUCTION

Greater sage-grouse (*Centrocercus urophasianus*; hereafter sage-grouse) was designated as a candidate species in March 2010 by the U.S. Fish and Wildlife Service (USFWS) for protection under the Endangered Species Act. In the 12-month finding, the USFWS determined that sage-grouse range wide warranted protection under the ESA but their listing was precluded because of higher conservation priorities.

Sage-grouse occupy sagebrush-steppe (*Artemisia* spp.) and require large expanses of sagebrush habitat. Sage-grouse currently occupy < 60% of the presettlement range, which includes 11 states and 2 Canadian Provinces. Declines in sage-grouse populations have mainly been attributed to habitat loss and degradation of the sagebrush-steppe ecosystem.

The Bear Lake Plateau and Valley (BLPV) sage-grouse population occurs at the edge of the Wyoming Basin in the southeastern subpopulation. Populations of sage-grouse at the edge of the range-wide distribution, such as the BLPV population, often depend on dispersal from connecting leks to sustain the genetic variation of these populations.

Because sage-grouse are capable of migrating considerable distances, the sage-grouse inhabiting my study area are believed to use habitats in three states. Pilot research conducted in 2010 confirmed this

belief, but the magnitude and importance of the interchange is uncertain (C.J. Cardinal, unpublished data). Obtaining this information could aid in the conservation of the BLPV sage-grouse population if the seasonal movements include multiple states where they are subjected to the jurisdiction of different state laws and management plans.

OBJECTIVES

1. Document population(s) vital rates of sage-grouse that inhabit the Bear Lake Plateau and Valley, Idaho, Utah, and Wyoming.
2. Document sage-grouse seasonal distribution and habitat-use patterns in the Bear Lake Plateau and Valley.
3. Determine if any differences observed in movement and habitat-use patterns are related to sex, age class, or land-use patterns.
4. Document how natural and anthropogenic land-use patterns and activities may contribute to habitat loss by fragmentation of sage-grouse habitats in the Bear Lake Plateau and Valley.

STUDY AREA

The Bear Lake Plateau and Valley Study Area (BLPV) consists of 207,500 hectares of area in Bear Lake County, Idaho, Rich County, Utah, and Lincoln County, Wyoming. The elevation of the study area ranges from 1,800 m to 2,500 m above mean sea level. The BLPV is comprised of many different land ownership and management entities but includes mostly private land, with some blocks of U.S. Forest Service, U.S. Fish and Wildlife Service, Bureau of Land Management, and state-owned land.

Vegetation is dominated by sagebrush-grassland plant communities. The main vegetation includes shrubs: *Artemisia* spp. *Chrysothamnus* spp.; grasses such as: *Agropyron cristatum*, *Bromus tectorum*, *Poa* spp.; and forbs such as: *Pholx* spp., *Agoseris glauca*, *Crepis acuminata*, *Baccharis salicina*, and *Antennaria rosea*. The climate of the study area is typical of intermountain highlands and is characterized by cold winters and hot summers. Temperatures range from lows of about -10°C in January, and highs of 29°C in July. The average precipitation is 36.1 cm, and the average annual snowfall is 104.4 cm (Western Regional Climate Center).

The primary land use is for grazing by domestic livestock. However, because of the presence of Bear Lake, the BLPV is a major seasonally recreation area, with most of the use occurring in summer. Additional residential development is occurring at the base of Bear Lake on both the east and west sides of the study area.

METHODS

Sage-grouse were trapped on and near leks beginning in March 2010 and additional birds were trapped through spring 2012. Spotlights were used to locate roosting grouse, and they were captured using a dip net, and fitted with radio-collars (Connelly et al. 2003). We attempted to capture and collar up to 40 male and 40 females with about half the collars deployed on grouse associated with leks along the Utah/Idaho border but in Utah and half in Idaho. We attempted to distribute them evenly on yearling and adults using size and plumage to classify grouse (Dalke 1963). Radio-collared grouse were located at least once a week from 1 April to 1 November and once a month from 1 November to 15 March 2012, depending on access.

Radio-collared females were located on nests by approaching and observing them under the same bush for several days but care is taken not to flush nesting birds. Nest success was measured by monitoring nest incubation time, and locating nest remains after success or failure. Brood success was determined by walking up females and counting the number of chicks, or by using night spotlighting.

Nest and brood vegetation was recorded beginning in 2011. A Robel pole was used to measure visual cover at nests, and four 15 meter line intercept transects at 90 degree angles from the nest were used to measure vegetation cover. Along these transects herbaceous cover was measured using Daubenmire frames. Aspect and the slope of the nest location were also recorded. Brood sites were measured using the line-intercept method at four 30 meter transects at 90 degree to measure shrub cover, and Daubenmire Frames were used to measure ground cover (grass, forb, bare ground, litter, rock) at 4 locations along these transects. Random vegetation points were taken for each nest and brood discovered to compare selected habitats to habitat points in the study area (Connelly et al. 2003).

Habitat fragmentation will be measured using GIS technology. Sage-grouse habitat use, production, and seasonal movements will be plotted relative to anthropogenic landscape features (Connelly et al. 2011). These metrics will be used to develop indices of habitat fragmentation to determine if the fragmentation observed constitutes functional habitat loss (USFWS 2010) or a possible barrier to movements. Sage-grouse movements will also be plotted relative to natural landscape barriers to determine how habitat-use is affected in this area.

RESULTS

2012 Research Progress

Captures

The 2012 snow melt came much earlier this year than the previous year. We were able to access the study site at the beginning of March to start trapping. The capture distribution from the 2012 trapping season can be found in Table 1. We captured 37 new birds- 13 females and 24 males. In addition, we captured 4 males with dead collars and recollared these as well. With the collars deployed, the season started with 47 cocks and 30 hens on air.

Table 1. The distribution of radio-collars deployed on greater sage-grouse in the Bear Lake Study Area during 2012.

Gender	Adult	Yearling
Bloomington (2B025) / Paris (2B003)		
Female	1	0
Male	5	4
Eden (2B014 and 2B015)		
Male	5	1
Indian Creek (2B042 and 2B043)		
Female	6	1
Male	3	2
Sheep Creek (2B032)		
Female	4	1
Male	7	1
TOTAL		
Female	11	2
Male	20	28

Leks

This spring we assisted Idaho Dept. of Fish and Game in their lek routes. We also investigated some leks that have not been observed in recent years. High lek counts can be found in Table 2.

Table 2. 2012 Lek Observations for the Bear Lake Valley and Plateau Study Area.

Lek	Date	Males	Females
2B002	04/07/2012	10	2
2B003	04/21/2012	23	0
2B012	03/29/2012	6	9
2B014	04/28/2012	43	7
2B015	04/28/2012	38	5
2B023	04/04/2012	0	0
2B024	04/04/2012	0	0
2B025	03/05/2012	39	2
2B032	03/29/2012	34	41
2B033	04/04/2012	0	0
2B038	04/25/2012	0	0
2B039	04/25/2012	0	0
2B040	04/25/2012	0	0
2B042	04/04/2012	16	6
2B043	03/29/2012	33	45

Nesting

At the beginning of the season, we started with 30 hens on air. As of 1 July, we have a total of 23 hens on air. A total of 19 nests were found during the 2012 spring and summer. Two hens are currently still nesting. Of the 17 completed nests, 7 were successful hatches and 12 were failures. Of the 7 dead hens, 4 were killed on nest, and 3 were killed post- nest failure. Of the nest failures it appeared that 5 depredations resulted from avian predators, and 7 depredations resulted from mammalian predators. As of 23 June, 6 of the 7 successful nesting hens were still observed to have chicks.

Movements

As during previous years, sage-grouse were found to move between states to different leks (Fig 1). This is mostly observed in males and females on the east side of the lake moving between Idaho and Utah. During 2012, sage-grouse were found to cross natural and anthropogenic barriers including Bear Lake, Bear River, highways, and residential areas. This spring, we observed our first sage-grouse to permanently relocate from the west side of the lake to the east side.

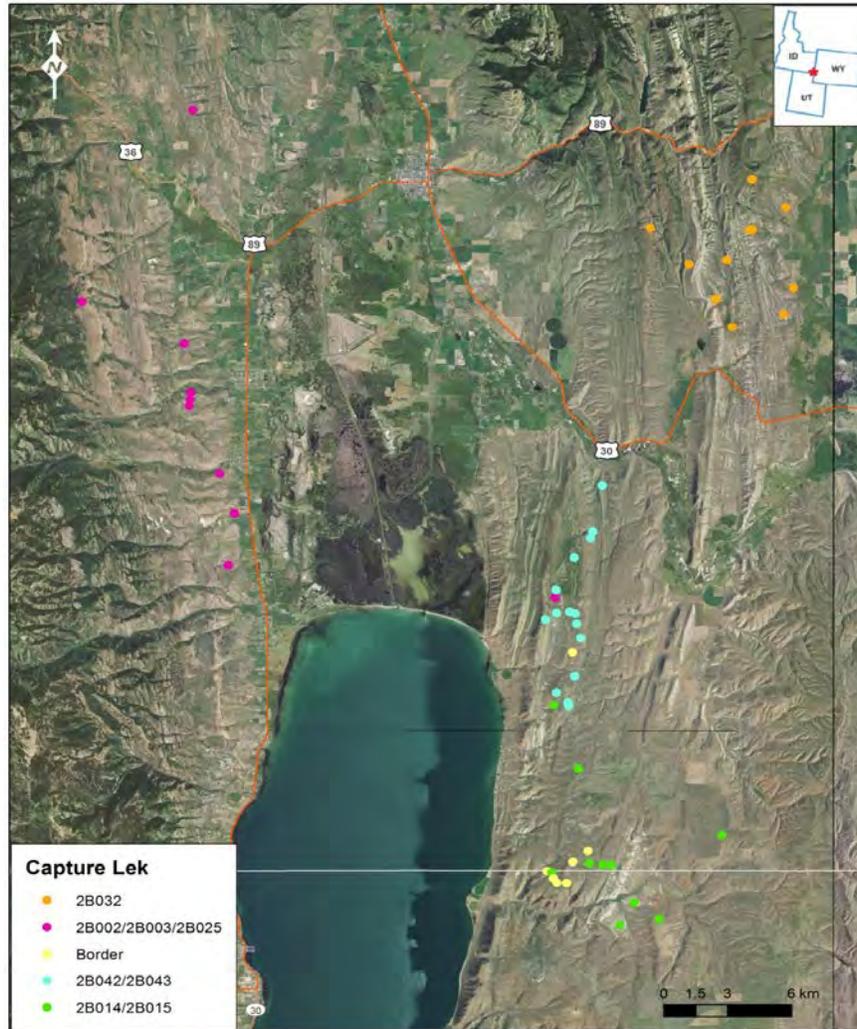


Figure 1. Movements of radio-marked sage-grouse on the Bear Lake Plateau, 2012.

Mortalities

During 2012, there have been 12 mortalities- 6 from mammalian predators (5 hens, 1 cock), and 6 from avian predators (2 hens, 4 cocks). The majority of the male mortalities happened during April and May when the cocks were in their breeding plumage. The hen mortalities occurred during May and June, and of the 7 hens killed, 4 mortalities occurred on nests. In addition to the mortalities, 3 collars were slipped by males during the spring.

Future Work Plan

The student has completed her field work and is planning on defending her thesis during fall 2013.

MANAGEMENT IMPLICATIONS

If sage-grouse in the Bear Lake Plateau and Valley Study Area do use habitat in all 3 states, this study will be useful for management cooperation between Idaho, Utah, and Wyoming. If birds are documented using habitat in the 3 states a conservation plan similar to the California-Nevada border plan could be constructed. This research will also be important to define the core use areas of valuable habitat for sage-grouse on the BLPV and thus help develop targeted conservation efforts in the future. If possible human impact could be reduced in vital breeding or wintering habitat, to promote sustainable populations in this area. Our results will also help define if or when migration of this population may occur. In future monitoring, this will aid in tracking birds, and studying habitat selection at different times of the year.

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A method for estimating population sex ratio for sage-grouse using noninvasive genetic samples

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Abstract

Population sex ratio is an important metric for wildlife management and conservation, but estimates can be difficult to obtain, particularly for sexually monomorphic species or for species that differ in detection probability between the sexes. Noninvasive genetic sampling (NGS) using polymerase chain reaction (PCR) has become a common method for identifying sex from sources such as hair, feathers or faeces, and is a potential source for estimating sex ratio. If, however, PCR success is sex-biased, naively using NGS could lead to a biased sex ratio estimator. We measured PCR success rates and error rates for amplifying the W and Z chromosomes from greater sage-grouse (*Centrocercus urophasianus*) faecal samples, examined how success and error rates for sex identification changed in response to faecal sample exposure time, and used simulation models to evaluate precision and bias of three sex assignment criteria for estimating population sex ratio with variable sample sizes and levels of PCR replication. We found PCR success rates were higher for females than males and that choice of sex assignment criteria influenced the bias and precision of corresponding sex ratio estimates. Our simulations demonstrate the importance of considering the interplay between the sex bias of PCR success, number of genotyping replicates, sample size, true population sex ratio and accuracy of assignment rules for designing future studies. Our results suggest that using faecal DNA for estimating the sex ratio of sage-grouse populations has great potential and, with minor adaptations and similar marker evaluations, should be applicable to numerous species.

Keywords: *Centrocercus urophasianus*, genetic sampling, greater sage-grouse, noninvasive molecular sexing, population sex ratio, sex ID

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Introduction

The sex ratio of free-ranging wildlife populations is an important metric for management and conservation as well as for understanding the evolutionary ecology of a species (Whittingham & Dunn 2000). Population sex ratios are useful for modelling offspring sex allocation and sex differences in survival (Donald 2007), are a necessary component for determining effective population size and modelling population viability (Lens *et al.* 1998; Sæther *et al.* 2004), and can be used as a metric of habitat suitability or food abundance (Wiebe & Bortolotti 1992; Stauss *et al.* 2005). Quantifying sex ratio is often difficult, especially for sexually monomorphic species, or for species where one sex is difficult to census (Donald 2007). Accurate estimates of sex ratio are further complicated

by differences in behaviour and ecology that exist between sexes such that most methods of sampling are sex-biased (Donald 2007).

Analysis of noninvasive genetic samples offers an alternative method for estimating the sex ratio of a population from hair, feathers or faeces that does not require capture or handling individuals (Waits & Paetkau 2005; Regnaut *et al.* 2006). The efficacy of DNA sexing techniques has been demonstrated for numerous species using feathers (Sacchi *et al.* 2004; Bush *et al.* 2005; Costantini *et al.* 2008; Gebhardt & Waits 2008), hair (Sloane *et al.* 2000; Banks *et al.* 2003; Fontanesi *et al.* 2008) and faeces (Robertson *et al.* 1999; Dallas *et al.* 2000; Segelbacher & Steinbrück 2001; Eggert *et al.* 2003; Idaghdour *et al.* 2003; Regnaut *et al.* 2006).

Due to the minute amount of DNA typically obtained using NGS techniques, errors from allelic dropout and PCR-generated false alleles are common (Taberlet *et al.* 1996; Broquet *et al.* 2007). To minimize the probability of

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assigning the wrong sex due to these problems, researchers have suggested techniques such as PCR replication using the multiple-tube approach and rigorous assignment criteria (Taberlet *et al.* 1996; Gagneux *et al.* 1997). When the primary objective is estimating sex ratio, a secondary source of error could be introduced by using a sex assignment method that is biased, (i.e. has a greater probability of accurately assigning one sex over the other). This occurs because the probability of allelic dropout may be higher for one sex than the other due to differences in fragment length or priming site mismatches and because confidence in assigning heterozygotes (females in the case of birds, males for mammals) is typically achieved with lower numbers of PCR replicates than confidence in assigning homozygotes (Taberlet *et al.* 1996).

Greater sage-grouse (*Centrocercus urophasianus*, hereafter sage-grouse) are an ideal candidate for using NGS to estimate population sex ratio. Sage-grouse have a lek mating system where males congregate on traditional display grounds each spring and females visit infrequently to select a mate and to copulate (Wiley 1978; Gibson & Bradbury 1986). Because of the conspicuousness of displaying males and the persistence of lek sites, number of males attending a lek or group of leks is commonly used as an index for detecting inter-annual population trends (Dalke *et al.* 1963; Connelly *et al.* 2000; Garton *et al.* 2011). However, since hens are considerably less conspicuous and their use of leks is much more variable than that of males (Gibson & Bradbury 1986; Schroeder *et al.* 1999), lek counts are ineffective for censusing females and therefore cannot be used to estimate sex ratios for sage-grouse.

In this study, we (1) determined PCR success rates and error rates for amplifying the W and Z chromosomes from faecal samples of sage-grouse using known sex samples, (2) examined how success and error rates for sex identification change in response to faecal sample exposure time in the field, and (3) used simulation models to determine how many PCR replicates are needed while evaluating bias and precision of 3 different sex assignment criteria for estimating population sex ratio for sage-grouse with various sample sizes.

Materials and methods

We collected sage-grouse faecal samples from birds of known sex that were radio-marked for a separate study. Sex of sage-grouse is easily distinguished by overall size and plumage (Dalke *et al.* 1963). This study was conducted on Browns Bench, Idaho, USA, which receives approximately 24 cm of precipitation annually and ranges in elevation from 1524 m to 2300 m. Major cover types include little sagebrush (*Artemisia arbuscula*)/black

sagebrush (*A. nova*)/grass, Wyoming big sagebrush (*A. tridentata wyomingensis*)/grass, mountain big sagebrush (*A. t. vaseyana*)/grass, mountain shrub and crested wheatgrass (*Agropyron cristatum*) seedings (Hironaka *et al.* 1983; Klott *et al.* 1993).

Field Sampling

We flushed 11 individual females and 7 individual males from roost sites in early morning of 12 February 2010, after the birds had deposited numerous faecal pellets in 'roost piles' over the course of the night. We chose to use faeces because sage-grouse defecate rectal faeces in a pellet form that, relative to feathers, are easy to locate for both sexes and not likely to be confused with that of other species. Location of each roost pile was recorded with a hand held GPS unit and marked with a pin flag to facilitate re-visitation. Sage-grouse roost on the ground in openings of sagebrush (Patterson 1952) and there is no evidence that they have any fidelity to a nightly roost site. Age of faecal pellets associated with each bird appeared similar and was assumed to be <1 day old. Sage-grouse have well developed ceca and defecate cecal faeces separately: these are easily distinguishable from the typical rectal faeces (Klasing 2005). For this study, we collected and analysed only rectal faeces. Faecal samples were placed in a coin envelope in an airtight plastic bag with silica until DNA extraction. A single faecal pellet was collected on the day birds were flushed (Time 0) and at the following time intervals after deposition: 2 days, 4 days, 6 days, 8 days, 15 days, 23 days, 34 and 124 days (18 June). Over this time, daily high temperatures ranged between 1.1 and 28.9 °C, daily low temperatures ranged between -8.8 and 13.3 °C, and a total of 11.7 cm of precipitation was measured 60 km from our field site at the Joslin Field-Magic Valley Regional Airport (National Climatic Data Service, NOAA 2012). Number of pellets was noted at time of each visit to ensure no new pellets had been added since day 0. Variation in faecal DNA degradation can depend on environmental factors such as precipitation and ambient temperature (Brinkman *et al.* 2010). For this study, we only considered the effects of time, so no weather covariates were considered.

DNA analysis

DNA was extracted in a room dedicated to low quantity DNA samples using a DNeasy Blood and Tissue kit (Qiagen, Inc.). We had compared this extraction technique previously to using the Qiamp DNA Stool Mini Kit (Qiagen, Inc.) and found a higher success rate with the tissue kit (unpublished data). We used 2–3 mm from each end of one pellet in the extraction. All samples were

amplified three times using a multiplex PCR that we developed to include two sets of fluorescently labelled sex ID primers 1237L/1272H (Kahn *et al.* 1998; hereafter Kahn) and 2550F/2718R (Fridolfsson & Ellegren 1999, hereafter Frid). The Frid primers amplify a portion of the CHD gene flanking an intron where the sex-dependent fragment (on the W chromosome) found only in females is shorter than the sex-independent fragment (on the Z chromosome) found in both males and females (W: 450 bp, Z: 600 bp). The Kahn primers amplify a different intron of the CHD gene, and the sex-dependent fragment is longer than the sex-independent fragment for most birds (Z: 240 bp, W: 270 bp for sage-grouse). We amplified all samples in a multiplex containing $3.5 \times$ Qiagen Master Mix (from the Multiplex PCR Kit), $0.5 \times$ Q solution, $0.14 \mu\text{M}$ each of Kahn primers and $0.57 \mu\text{M}$ each of Frid primers. PCR conditions started with a 95°C denaturation step for 15 min, followed by 45 cycles of 94°C for 30 s, $51\text{--}48^\circ\text{C}$ for 90 s (stepping down 0.5°C per cycle until reaching 48°C) and 72°C for 30 s, and a final annealing step of 60°C for 30 min. PCRs were performed with a Tetrad 2 DNA Engine thermal cycler (Bio-Rad, Hercules, CA, USA) and PCR products were separated with a 3130xl Capillary Sequencer (Applied Biosystems, Foster City, CA, USA) and visualized using GENEMAPPER 3.7 (Applied Biosystems, Foster City, CA, USA).

We estimated PCR success rates and genotyping error rates for each primer set across all time points. PCR success rates were estimated for males and females combined as the number of successful amplifications for each primer set divided by the total number of PCRs attempted for each primer set (Broquet & Petit 2004). We estimated allelic dropout error rates for females as the failure to amplify the W or the Z chromosome divided by number of successful amplifications and we estimated the false allele error rates for males as the number of times the W chromosome amplified for a male sample out of the total number of successful PCRs (Broquet & Petit 2004). We tested for a linear relationship between total PCR success and days of exposure. Since PCR data consists of binary responses, we used logistic regression with Generalized Estimating Equations (Liang & Zeger 1986), treating each sample from the same faecal pile as a repeated observation.

We evaluated sex assignment success rates and resulting sex ratio estimates across all time points from the three replicates of the two primer sets using three possible criteria for assigning sex ID. Criterion 1 required two Z/W or W chromosome results to assign the sample as female and three Z chromosome results to classify a male. The basis of this criterion is that the weight of evidence to confirm a heterozygote is less than that required to confirm a homozygote because allelic dropout is a much more common artefact of NGS analysis

than generation of a specific false allele (Gagneux *et al.* 1997), and is often used in NGS studies for this reason (e.g., Stenglein *et al.* 2011). However, this conservative criterion might lead to a skew in estimated sex ratios because success rates for females (requiring two positive PCRs) could be higher than success rates for males (requiring three successful PCRs). Criterion 2 required two Z/W or W chromosome results to classify as female and two Z chromosome results for male. This criterion was designed to ensure accuracy through duplication, but attempts to equalize opportunity for successful identification of males and females. Criterion 3 required a single Z/W or W chromosome result to classify as female and only a single Z chromosome result for male. The third criterion was the least conservative and was designed to maximize the number of samples assigned one sex or the other and thereby increase sample size used to estimate sex ratio. As a tradeoff, this criterion has a higher likelihood of misclassifying females as males when the W chromosome drops out. All results that did not meet requirements for a given criterion were considered inconclusive and were not used for estimating the sex ratio.

We calculated sex assignment success as the proportion of all the samples that were assigned a sex for each of the three criteria. Estimated sex ratio was determined from number of samples successfully assigned either sex and expressed as proportion female estimated.

Simulations

We simulated a population of sage-grouse faecal samples to investigate the effects of sample size and true sex ratio on the efficacy of the three sex assignment criteria for estimating sex ratio. We used the frequencies of results for each sex from the first eight observations (day 0–day 34) of the 18 roost piles to estimate the probabilities of PCR amplification for our simulations. We treated the repeated measures through time as independent samples and considered the three PCR replicates for each primer set and the results from the two primer sets as independent. While there may be some autocorrelation between loci amplified in the same reaction, the high level of efficiency of the enzymes used in this reaction should have minimized this effect. We simulated samples with a balanced sex ratio (1:1), modestly skewed sex ratios of 1:1.25 in favour of both males and females, and a more extreme skew of 1:2.5 in both directions using sample sizes of 50, 100, 150, 300 and 500, and replicated each simulation 5000 times. We analysed each sex assignment criterion with three PCR replicates, and also analysed the second and third assignment criteria with only two PCR replicates for both primer sets. For each combination of sex ratio, sample size and number of replicates, we simu-

lated the sampling distribution of estimated sex ratio, and calculated the mean, mean bias and mean variance for the estimated sex ratio for each assignment criterion. Simulations were performed in program R (R Development Core Team 2009).

Results

DNA analysis

PCR success rates for both primers for the first eight time periods were higher for females than males and greater for the Kahn primers than the Frid primers (Table 1). Average success with Kahn primers for collection intervals 1–8 were 83% and 76% for female and male, respectively, and with Frid primers were 44% and 23% for female and male respectively. There was no evidence of a linear trend in total PCR success from day 0 to day 34 (slope = 0.015, SE = 0.019, $Z = 1.23$, $P = 0.218$); however, success dropped to 0 for both sexes with the Frid primers and 15% and 14% for female and male, respectively, with the Kahn primers at day 124. Average PCR error rates for failure to amplify the W allele in females over the first eight collection intervals were the same for both primer sets (15%, Table 1). Failure to amplify the Z allele in females was greater with the Frid primers than with the Kahn primers (57% and 14%, respectively). False W allele amplification was not observed for males with either of the primer sets used for any collection period.

Success rates for sex assignment were at or above 50% for all three criteria at all time points except at $t = 124$ days and did not show a decrease over time through day 34 (Fig. 1). Average sex assignment success rates using the more stringent Criterion 1 were 66% (range 6–89%) and were highest at 34 days. When using Criterion 2, average sex assignment success rates increased to 73% (range 11–94%) and were highest at 15 days. Average sex assignment success rates using Criterion 3 were highest, averaging 83% (range 28–100%),

and reaching 100% at 34 days. No samples were misclassified using Criterion 1, and only one sample was misclassified for Criterion 2 (one female misclassified as male from samples with 124 days of exposure). The third criterion resulted in six misclassifications of females as males; one with 2 exposure days, one with 4 exposure days and four with 124 exposure days.

The estimates of sex ratio at each time point typically differed from the true sex ratio (0.611) because some samples failed to amplify (Fig. 1). When using Criterion 1, the estimates from days 0–34 were biased towards females and were on average 0.029 higher than the true proportion (Table 2). When using Criterion 2, the average bias across samples from days 0–34 was 0.010, but biased towards males. The average bias across these same time points was <0.001 for the third criterion and was also biased towards males.

Simulations

We used our empirical marker frequency results from 862 observations (18 birds \times 8 repeated samples \times 2 primers \times 3 typing replicates, less 1 typing replicate for each primer for 1 female that was not run; Table 3) as multinomial probabilities to generate simulated sage-grouse faecal sample populations. Criterion 1 disproportionately failed to assign sex to faecal samples from male sage-grouse at all sample sizes and sex ratios, resulting in female-biased estimates (Fig. 2). Because Criterion 1 was the most conservative set of decision rules, this method consistently used the least number of sex-assigned samples to estimate the sex ratio and resulted in the largest standard errors, which were exceptionally large with relatively small samples sizes ($n \leq 200$).

Both Criteria 2 and 3 resulted in more accurate estimates of sex ratio than criterion 1 under all simulated conditions. When only two PCR replicates were used, Criterion 2 resulted in sex ratio estimates that were similar in precision to estimates from Criterion 1 with

Table 1 PCR success and error rates for both primer sets and sexes at nine exposure times from greater sage-grouse faecal samples collected on Brown's Bench, Idaho, USA, 2010. First number in each column refers to the Fridolfsson and Ellegren (1999, Frid) primers; second number refers to the Kahn *et al.* (1998) primers. False amplification of the W allele was not observed for male samples.

	Exposure days																		Average (day 0-34)	
	0	2	4	6	8	15	23	34	124											
Female																				
Success	0.52	0.85	0.33	0.76	0.48	0.85	0.27	0.82	0.18	0.61	0.64	0.97	0.53	0.97	0.55	0.85	0.00	0.15	0.44	0.83
W dropout	0.18	0.18	0.09	0.20	0.13	0.18	0.22	0.33	0.00	0.10	0.19	0.06	0.29	0.06	0.11	0.11	NA	1.00	0.15	0.15
Z dropout	0.47	0.07	0.45	0.12	0.50	0.14	0.56	0.22	0.67	0.30	0.57	0.03	0.65	0.16	0.67	0.07	NA	0.00	0.57	0.14
Male																				
Success	0.48	0.71	0.29	0.95	0.24	0.71	0.05	0.67	0.19	0.76	0.10	0.76	0.05	0.52	0.43	0.95	0.00	0.14	0.23	0.76

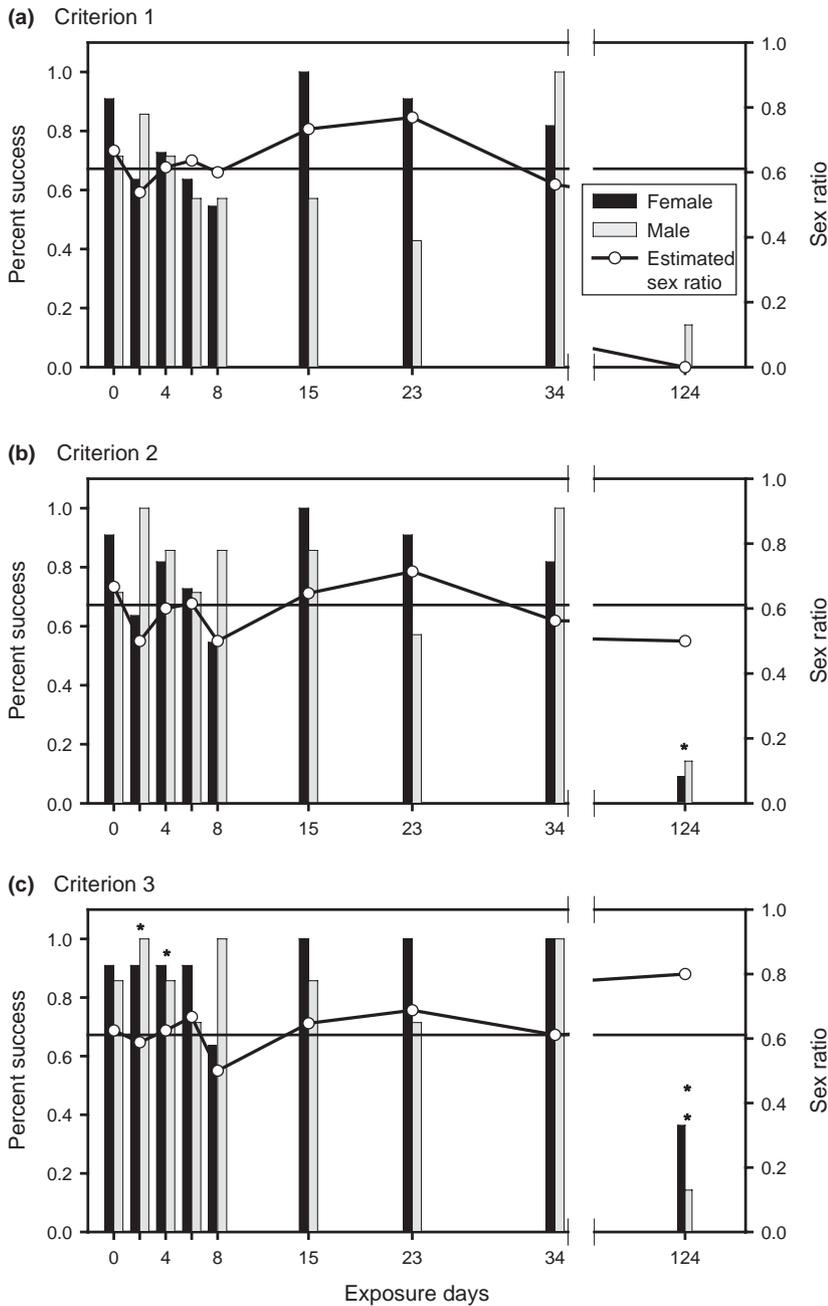


Fig. 1 Sex assignment success and associated sex ratio estimate for each of nine collection occasions for (a) Criterion 1, (b) Criterion 2 and (c) Criterion 3 from greater sage-grouse faecal DNA collected on Brown's Bench, Idaho, USA, 2010. Single asterisk indicates a single sample falsely identified as male. Two asterisks indicate four samples falsely identified as male. Horizontal line represents actual sex ratio (0.611).

three replicates, and slightly female biased under all but the most extreme female-skewed populations simulated. Criterion 3 resulted in slight male-biased sex ratio estimates under all but the most extreme male-skewed populations simulated. Increasing the number of PCR replicates from 2 to 3 increased the number of potential results evaluated by the various decision rules from 4 to 6, which reduced the bias and increased precision regardless of the criterion used (Figs. 2B & 2C).

Criteria 2 and 3 produced relatively unbiased estimates of sex ratio at all sample sizes and sex ratios

simulated. Because Criterion 3 was the least conservative, this method used the largest number of sex-assigned samples to estimate sex ratio and produced the most precise estimates (i.e. relatively smallest variance).

Discussion

We demonstrate how sex ratios can be accurately estimated for greater sage-grouse populations from noninvasive genetic samples despite sex bias PCR success. With minor adaptations, the methods we outlined above

Table 2 Sex assignment success and associated sex ratio estimates for three sex assignment criteria for sage-grouse faecal DNA collected on Brown's Bench, Idaho, USA, 2010. Data were combined across time from day 0 through day 34. True sex ratio was 0.611.

	% success			Estimated sex ratio*
	Female	Male	Total	
Criteria 1	77.3	67.9	73.6	0.640
Criteria 2	79.5	82.1	80.6	0.601
Criteria 3	90.9	87.5	89.6	0.619

*Sex ratio calculated as proportion of all samples assigned a sex that were assigned female.

should be applicable to numerous species, adding to the limited number of existing tools for estimating population sex ratio. PCR amplification was relatively successful (>75% with the Kahn primers) for sage-grouse faecal samples with ≤ 34 exposure days and we did not detect a decrease in success with exposure time during this period. This indicates a slow rate of DNA degradation in these samples, potentially resulting from dry conditions and/or the diet of this species, and differs from results showing PCR success rates dropping rapidly for mammals under more humid conditions (Murphy *et al.* 2007; Brinkman *et al.* 2010). These results are encouraging since field-collected faecal samples of unknown origin can only be qualitatively dated as fresh or not.

PCR success was greater with the Kahn primers than the Frid primers, as was expected considering the PCR product size amplified by each primer set and the degraded quality and low quantity of DNA typical of noninvasive samples (Buchan *et al.* 2005). Despite the relatively poor success of the Frid primers, we chose a multiplex approach for our analysis since doing so doubled the number of potential outcomes to evaluate for sex assignment from 3 to 6 with minimal increase in cost. A multiplex approach is a common approach for improving efficiency for species identification (Dalen *et al.* 2004; Mukherjee *et al.* 2007) and individual identification (Jacob *et al.* 2010; Stenglein *et al.* 2011) of NGS, but it is rarely used in sex ID (Gebhardt & Waits 2008).

Our use of the three sex assignment criteria resulted in sex ratio estimates that differed in bias and precision. PCR success was lower for males than females for both primer sets which, when combined with the more conservative sex assignment rule for males than for females in Criterion 1, resulted in female-biased sex ratio estimates (Table 2). The sex assignment rules for Criterion 2 greatly reduced the effects of the biased PCR success, resulting in relatively unbiased sex ratio estimates. The least conservative assignment rules of Criterion 3 resulted in the largest proportion of the sample of both males and females assigned a sex, and the greatest

probability of falsely assigning females as males. Under the conditions we simulated with three PCR replicates, the erroneous assignments seemed to offset the female-biased PCR success, resulting in relatively unbiased sex ratio estimates with the greatest precision.

With birds, identification of the homozygous male may be a factual result from male DNA or the result of failure to amplify the female 'W' gene. Increasing genotyping replication increases the probability of W allele amplification for females and thus reduces the probability of assignment errors (Lynch & Brown 2006). In our simulation example, increasing from 2 to 3 typing replicates decreased the proportion of female samples that were falsely assigned as males, reducing the slight male bias of Criterion 3 (Fig. 2C). The tradeoff to typing replication is an increase in costs of approximately 25% for each additional replicate beyond 2 due to increased time and products required.

Our study is an important first step towards developing a noninvasive method for estimating population sex ratios for sage-grouse. Further research is needed to examine the rate of degradation of faecal DNA under various environmental conditions and over the 1–4 month time period to verify that the sex bias in PCR success that we observed does not change under different field conditions and longer exposure times. In addition, if this method was considered to estimate sex ratio of brooding young, potential effects of the differing diet of sub-adults on the rate of degradation of faecal DNA would also need to be considered since other studies have shown an effect of diet on faecal DNA amplification success (Murphy *et al.* 2007; Panasci *et al.* 2011).

We used faecal samples collected in winter because at this time sage-grouse are commonly found in mixed-sex flocks (Beck 1977; Connelly 1982) and both sexes use the same areas for foraging and roosting, regardless of flock composition. To use the method we described for estimating population sex-ratio, a study design that produces a random sample of faecal pellets is necessary. For sage-grouse, we suggest collecting faecal pellets along predefined transect lines established through wintering grounds. Coordinating collections to immediately follow snow events should reduce the likelihood of collecting multiple samples from the same bird, as well as ensuring a relatively high quality of DNA if only faecal samples from on top of the snow are collected. To further reduce the likelihood that individual birds are represented multiple times in the sample, single pellets from roost piles could be collected rather than collecting individual pellets that are defecated singularly as the birds forage.

Additional information with regard to faecal deposition rates for males and females would improve estimates when relating a sex ratio of faecal samples to an actual population sex ratio. The assumption of equal

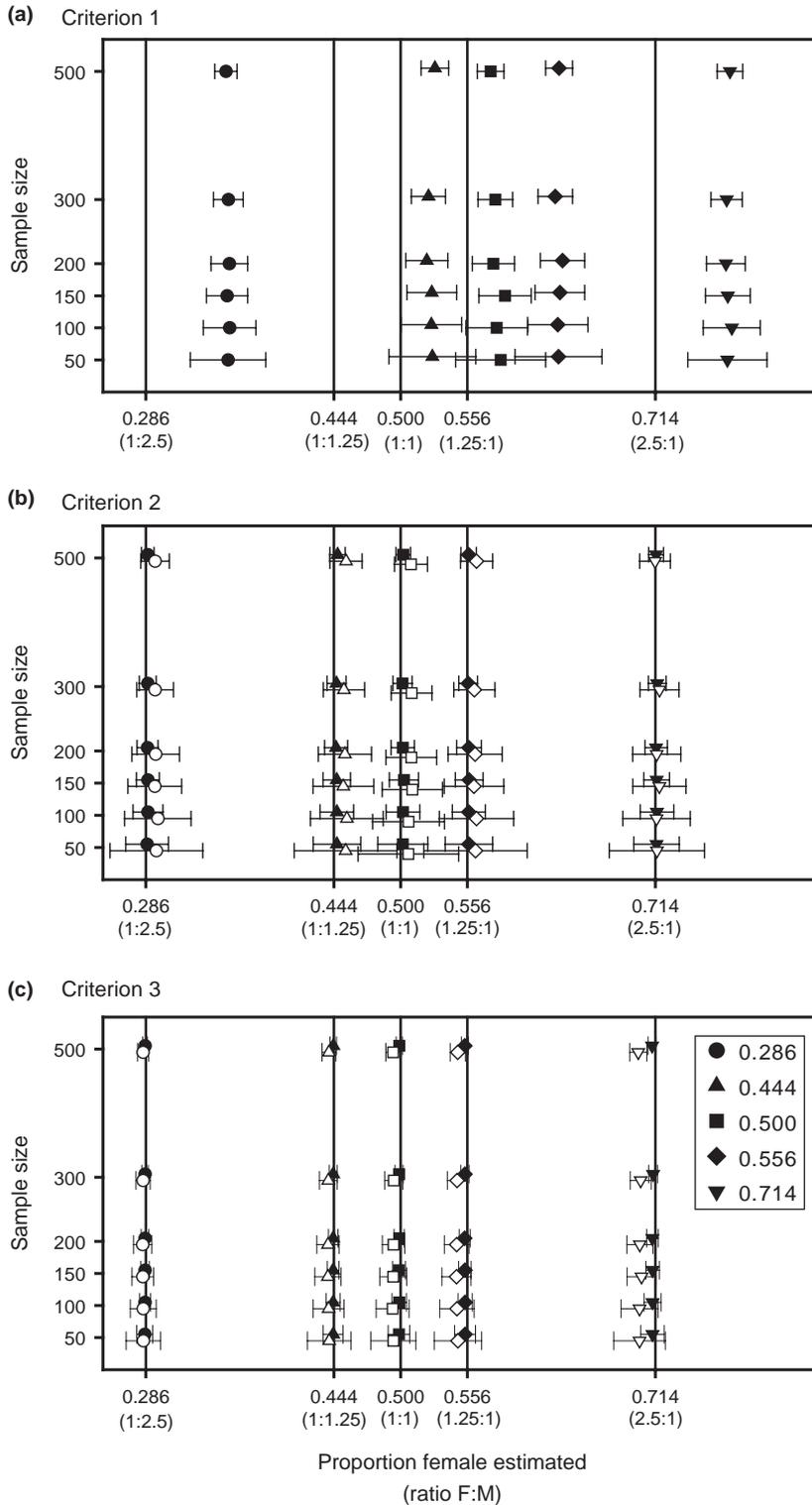


Fig. 2 Estimated greater sage-grouse sex ratio and associated variance for sex assignment criterion: (a) Criterion 1, (b) Criterion 2 and (c) Criterion 3 evaluated at six different sample sizes and five sex ratios from simulated data. Error bars represent ± 1 standard deviation about the mean. Solid black symbols represent three replicates, hollow symbols represent two replicates and vertical lines represent the true sex ratios associated with each simulation.

deposition rates between the sexes may not be valid for sage-grouse considering the nearly 2 to 1 size dimorphism of males compared with females (Schroeder *et al.* 1999). Individual identification by molecular typing from

a large sample of faeces could be used to test the assumption of equal faecal deposition rates (Kohn *et al.* 1999; Ebert *et al.* 2010; Jacob *et al.* 2010). These data could be further analysed under a mark-recapture framework

Table 3 Primer and sex-specific marker results used to create simulated populations from sage-grouse faecal samples collected on Brown's Bench, Idaho, USA, 2010.

	Kahn			Fridolfsson and Ellegren (Frid)		
	Result	Frequency	Probability	Result	Frequency	Probability
Female	WZ	158	0.601	WZ	30	0.114
	WW	28	0.106	WW	65	0.247
	ZZ	33	0.125	ZZ	20	0.076
	null	44	0.167	null	148	0.563
Male	ZZ	127	0.756	ZZ	38	0.226
	null	41	0.244	null	130	0.774

such that detection probabilities between the sexes could be used to estimate differences in faecal deposition rates (Lukacs & Burnham 2005). The efficacy of the method we describe also relies on the assumption that there is no heterogeneity in habitat use between the sexes at the sampling scale (Dallas *et al.* 2000), which will require scrutiny of individual populations.

Application of this method for other species is possible with some key modifications. These include: identifying one or more sex ID primers for the species of interest, estimating the sex bias in PCR success and developing sex assignment criteria and level of replication that results in unbiased estimates of sex ratio from known sex samples, and sampling from a space and time that both sexes use equally.

The results from our analysis of field-collected sage-grouse faeces in winter and spring and our simulations suggest that estimating the sex ratio of wild populations using faecal DNA has great potential; however, failing to account for the potential sex bias in PCR success can lead to greatly biased sex ratio estimates. Our simulations demonstrate the importance of considering the interplay between the sex bias of PCR success, number of genotyping replicates, sample size, true population sex ratio and accuracy of assignment rules for designing future studies. Furthermore, the research goal of a study where sex ratio is estimated has bearing on the study design. For example, if detection of a difference among sex ratios is desired, maximizing precision and therefore power is advantageous. If, however, sex ratios will be used to relate known numbers of one sex to unknown numbers of the other sex, methods minimizing the bias in sex ratio estimates should be sought in exchange for precision and/or cost. Alternatively, our methods outlined above could be used to develop an unbiased estimator of sex ratio by estimating and correcting for bias with any single set of criteria.

Continued development of estimating sex ratio from faeces will hopefully improve our understanding of sex ratios of sage-grouse populations. This approach to

estimating population sex ratios using species-appropriate makers, may prove to be useful for populations of other species of galliformes and terrestrial anseriformes that typically produce faeces in a pellet form, as well as most mammals, provided any bias in defecation rates or detection rates between the sexes is accounted for. Other forms of noninvasive DNA could also be used, such as hair or feathers (Taberlet *et al.* 1999; Waits & Paetkau 2005), to produce an unbiased estimator of population sex ratios for an even greater number of species.

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Data Accessibility

Sex identification primer results: DRYAD entry doi:10.5061/dryad.v0q5p.

Effects of Landscape-Scale Environmental Variation on Greater Sage-Grouse Chick Survival

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Abstract

Effective long-term wildlife conservation planning for a species must be guided by information about population vital rates at multiple scales. Greater sage-grouse (*Centrocercus urophasianus*) populations declined substantially during the twentieth century, largely as a result of habitat loss and fragmentation. In addition to the importance of conserving large tracts of suitable habitat, successful conservation of this species will require detailed information about factors affecting vital rates at both the population and range-wide scales. Research has shown that sage-grouse population growth rates are particularly sensitive to hen and chick survival rates. While considerable information on hen survival exists, there is limited information about chick survival at the population level, and currently there are no published reports of factors affecting chick survival across large spatial and temporal scales. We analyzed greater sage-grouse chick survival rates from 2 geographically distinct populations across 9 years. The effects of 3 groups of related landscape-scale covariates (climate, drought, and phenology of vegetation greenness) were evaluated. Models with phenological change in greenness (NDVI) performed poorly, possibly due to highly variable production of forbs and grasses being masked by sagebrush canopy. The top drought model resulted in substantial improvement in model fit relative to the base model and indicated that chick survival was negatively associated with winter drought. Our overall top model included effects of chick age, hen age, minimum temperature in May, and precipitation in July. Our results provide important insights into the possible effects of climate variability on sage-grouse chick survival.

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Introduction

Selective pressures result in the evolution of a life history conducive to species persistence under the environmental conditions encountered throughout the species' evolutionary history. Environmental conditions are not static, but rather experience climatic, geological, and successional changes through time. While such changes continue to occur naturally, anthropogenic disturbances have critically altered many of these processes, resulting in environments changing at rates that exceed the ability of some species to adapt [1]. The impact of rapidly changing environments may be particularly severe for species with limited dispersal opportunities (i.e., those existing in highly fragmented habitats; [2]). Efforts to conserve such species must focus on identifying the key demographic rates that are limiting population growth and the environmental factors that affect these rates [3].

During the 20th century, greater sage-grouse (*Centrocercus urophasianus*; hereafter sage-grouse) populations experienced precipitous declines as a result of anthropogenic habitat destruction, degradation, conversion, and fragmentation [4,5]. In response to

declining populations and increasing threats to remaining habitat, the Canadian Committee on the Status of Endangered Wildlife in Canada declared sage-grouse to be an endangered species in 1998 [6]. The United States Fish and Wildlife Service (USFWS) designated the sage-grouse as a candidate for protection under the Endangered Species Act in 2010 [7].

Sage-grouse are endemic to sagebrush (*Artemisia* sp.) dominated habitats of western North America, which have historically been very stable given that sagebrush is a long-lived and persistent plant. As such, sage-grouse evolved to use sagebrush for food and cover throughout the majority of their annual cycle. However, sage-grouse chicks do not consume sagebrush during their early development but instead require forbs and their associated arthropod communities. These components of the sagebrush ecosystem are highly dependent upon precipitation levels and therefore may exhibit high interannual variability. Thus, sage-grouse evolved a life history characterized by high annual adult survival but relatively low and variable reproductive rates compared to most other tetrapods [8,9].

Recently, researchers have applied life cycle models to gain a better understanding of factors affecting greater sage-grouse at the population [10] and range-wide scales [9]. Although both studies found sage-grouse population growth rates to be most sensitive to variability in adult female survival, they also found chick survival to have the second largest impact on population growth. While numerous studies have evaluated factors which influence survival rates of adult female sage-grouse [11,12], little is known about factors affecting chick survival. Generally, demographic rates to which the population growth rate is highly sensitive have low temporal variability [13,14]. Thus, chick survival should exhibit greater inter-annual variability and could therefore contribute more to spatio-temporal variability in population growth rate [15] even though sage-grouse populations are more sensitive to hen survival.

Previously published studies of factors affecting sage-grouse chick survival [16,17,18] have focused on micro-scale habitat factors such as percent coverage and height of forbs and grasses and availability of arthropods at chick location sites. These studies follow logically from previous research on sage-grouse brood habitat selection [19,20,21,22] and chick diets [23,24,25,26]. Collectively, these studies clearly demonstrate that broods typically select relatively mesic habitats with abundant forbs and arthropods and that these choices are related to chick survival. However, existing studies have not investigated the impacts of large-scale environmental processes (drought, temperature, etc.) on sage-grouse chick survival.

Landscape-scale environmental factors such as habitat condition, drought, and climate may be correlated with chick survival. Normalized Difference Vegetation Index (NDVI) is a commonly used index of plant production and habitat quality [27,28,29,30], with higher values of the index corresponding to increased levels of “greenness”. Despite being less sensitive to plant phenology in sagebrush steppe ecosystems [29] and potential biases due to image quality, NDVI has been shown to be positively related to sage-grouse recruitment and population growth [27]. Drought and climatic variables can work independently and in concert to affect habitat parameters and can be reflected in NDVI values. For example, measures of drought, precipitation, and temperature can be correlated to winter snow pack which is known to be a major driver of vegetation dynamics throughout much of the mountainous regions of western North America [31]. However, climatic variables may affect sage-grouse chick survival in ways other than through their influence on habitat quality. Young grouse may be susceptible to exposure mortality during periods of extreme temperatures [32]. Additionally, numerous studies have documented increased nest and chick predation rates following precipitation events (i.e., moisture facilitated predation hypothesis; [33,34,35]). This effect is typically attributed to increased scent production resulting from increased bacterial growth when skin and feathers are wet [36]. Although the assumptions underlying the moisture facilitated predation hypothesis have not been thoroughly evaluated in the context of the hypothesis, the processes of moisture facilitating microbial activity and increased microbial activity resulting in increased scent production have been well documented in other fields of study [37,38,39,40].

The objective of our study was to model the effects of landscape scale biotic (habitat greenness) and abiotic (climate and drought) factors on sage-grouse chick survival. We demonstrate the utility of data that can be readily obtained for virtually any geographic region or temporal period via web-based resources for predicting sage-grouse chick survival.

Materials and Methods

Study areas

Data were collected as part of 2 larger studies conducted in Idaho and Utah (Fig. 1). The Idaho study was conducted from 1999–2002 in sagebrush-grassland habitats of the Upper Snake River Plain in southeastern Idaho (44°13'N, 112°38'W). This area was characterized by relatively low topographic relief with elevation across the site ranging from 1300–2500 m. Approximately 50% of the area was privately owned, with the remainder being public lands administered by the U.S. Bureau of Land Management (BLM). Annual precipitation varied by elevation with low elevation areas receiving 17.5 to 30.0 cm of precipitation. Most sage-grouse habitat at lower elevations was dominated by Wyoming big sagebrush (*A. tridentata wyomingensis*). At higher elevations precipitation ranged from 30.5 to 45.5 cm annually and the habitat was dominated by mountain big sagebrush (*A. t. vaseyana*). Livestock grazing and cropland agriculture were the dominant land uses across the area [41].

The Utah study area was located on Parker Mountain in south-central Utah (38°17'N, 111°51'W). Research on sage-grouse chick survival was conducted on this site from 2005 through 2009. The area encompassed 107,478 ha and was administered by the Utah School and Institutional Trust Lands Administration (40.8%), United States Forest Service (20.2%), BLM (33.9%), and private ownership (5.1%). Parker Mountain is a sagebrush-dominated plateau at the southern edge of the sage-grouse range. It is one of the few areas remaining in Utah with relatively stable numbers of sage-grouse, and it includes some of the largest contiguous tracts of sagebrush in the state [42]. Grazing by domestic livestock is the predominant land use practice across the study site. The area receives between 40 and 51 cm of precipitation annually, which generally exhibits a bi-modal pattern, occurring either as rain during the seasonal monsoonal period from late summer and early autumn or as snow during winter.

Field methods

We captured female sage-grouse on and around leks using spotlights, binoculars and long handled nets [43,44] during early spring (March–April). Captured hens were classified as being either second-year (SY) or after second-year (ASY) birds based on wing characteristics as described by Beck et al. [45]. Birds were fitted with 15–19 g necklace style radio-transmitters (Advanced Telemetry Systems, Isanti, MN, USA; Holohil Systems, Carp, Ontario, Canada) and released at the capture location.

Marked hens were monitored during April and May to determine if they initiated a nest. Nesting was confirmed visually, but hens were never intentionally flushed from their nest due to the tendency of female sage-grouse to abandon their nest if disturbed [46,47]. Nesting hens were visually monitored every 2–3 days to determine nest fate. Nests were monitored daily as the anticipated hatch date approached.

We captured chicks by using telemetry equipment to locate radio-marked hens. During capture events, the brood hen was flushed and chicks were captured by hand and placed in an insulated container to help maintain body temperature. We captured most broods within 48 hours of hatching with all broods being captured within 1 week of hatching. Captured chicks were weighed to the nearest gram and marked with a ≤ 1.5 g backpack-style radio-transmitter (Advanced Telemetry Systems, Isanti, MN in 1999–2001 and 2005, Holohil Systems, Carp, Ontario, Canada in 2006–2008, and American Wildlife Enterprises, Monticello, FL in 2009) attached with 2 sutures [48]. For the Idaho study site, 2–3 chicks per brood were selected at random to receive radio-

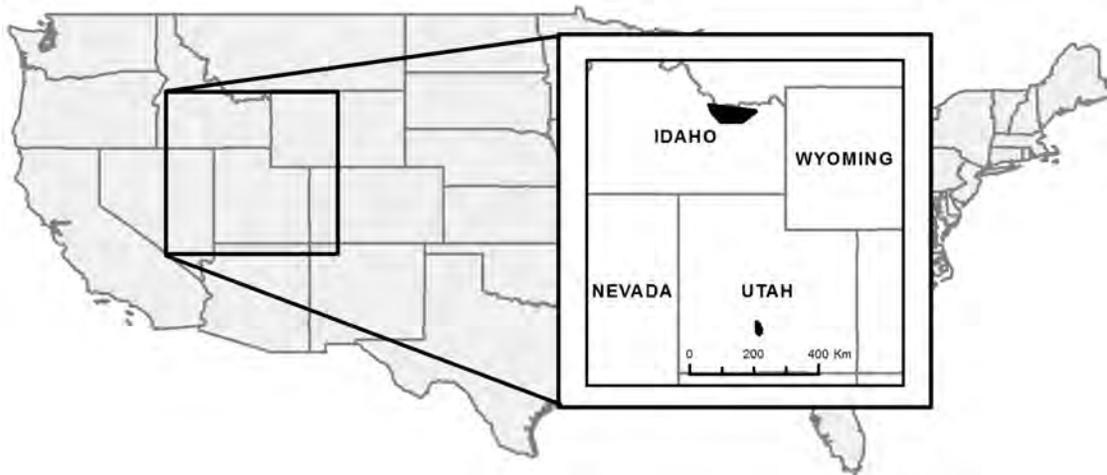


Figure 1. Map of study areas in Idaho and Utah.
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transmitters. At the Utah study area, we marked all captured chicks except in 2006 when 3 chicks from each brood were randomly selected to receive transmitters. Chicks found dead in the immediate vicinity of the capture site were considered to have died as a result of handling and were excluded from subsequent analyses. Broods were typically checked within 12 hours of being marked and all chicks classified as capture mortalities were found intact within a few meters of the release site, indicating that their death was directly attributable to the capture event. Our decision to exclude chicks classified as capture mortalities from our analysis may have inflated survival estimates if some of these mortalities were in fact not related to capture. However, we do not believe this was a common occurrence if it occurred at all.

Marked chicks were located every 1–2 days until they reached 42 days of age. Monitoring intervals did occasionally exceed 2 days due to inclement weather events or difficulties locating broods following large movements. Extensive efforts were made to find any chicks missing from a brood. We occasionally recovered chick transmitters with no chick remains or signs of predation. These recoveries were classified as mortalities although it is possible that transmitters may have been lost for reasons other than chick death. Alternatively, we could have right-censored these specific events. While this would have been a valid option, we chose to treat the events as mortalities to ensure that our survival estimates were conservative. Due to the difficulty of distinguishing predation from scavenging, we did not assign specific causes of mortality.

All necessary permits were obtained for the described field studies. Permission to capture and mark sage-grouse in Idaho was obtained from the Idaho Department of Fish and Game and from the Utah Division of Wildlife Resources for the Utah study site. Grouse capture and transmitter attachment procedures were approved under the Utah State University Institutional Animal Care and Use Committee (IACUC) permit #945R and #942 and University of Idaho IACUC permit #2000-7.

Covariate data

We compiled year and site specific covariate data pertaining to drought, landscape greenness, and climate. We included seasonal (preceding winter and current summer) and monthly (May–July) Palmer Drought Severity Index (PDSI) and Palmer Z-Index (PZI) values. For climate and drought covariates, we defined winter as the period from 1 November to 30 April because precipitation

would likely fall as snow on both study sites during these months. Summer was defined as the period from 1 May through 31 July. We did not include August because very few broods were monitored beyond July.

While both the PDSI and PZI indices are measures of drought and their values interpreted similarly (negative values correspond to drought conditions while positive values indicate wet conditions), the PDSI is most appropriate for measuring conditions across long time periods (several months) while the PZI is designed to measure conditions across shorter time periods (several weeks to a few months, [49]). Although drought is often thought about in terms of the presence or absence of precipitation, PDSI and PZI also account for site specific rates of evapotranspiration, soil moisture recharge, runoff, and moisture loss [50]. Additionally, both drought indices are calculated relative to the long-term average drought conditions at a specific site. As such, values of each index are standardized to have a common interpretation across locations [50]. All drought data were downloaded from the National Oceanic and Atmospheric Administration's National Climate Data Center (<http://www.ncdc.noaa.gov/temp-and-precip/time-series/index.php>).

Climate variables of interest included total precipitation, minimum temperature, and maximum temperature for the same seasonal and monthly periods described above. Unlike drought covariates, climate covariates were not adjusted to account for other physical processes or long-term site-specific averages. Because complete and representative weather station data were not available for both study sites, we used the Parameter-elevation Regressions on Independent Slopes Model (PRISM; <http://www.prism.oregonstate.edu/>) to estimate climatic data for both sites. PRISM is a knowledge-based climate analysis system capable of generating gridded predictions of climate data from known point climate data and a digital elevation map [51]. We used ArcMap10 to generate minimum convex polygons around all chick locations at both sites to define our study sites. We then extracted climate variable data from the corresponding PRISM layer.

Phenological change in landscape greenness was measured using NDVI for each study area. We generated NDVI values using Landsat 4–5 satellite images obtained from the United States Geological Survey EarthExplorer website (<http://earthexplorer.usgs.gov/>). We selected images captured between 1 May and 31 August with minimal cloud coverage. Due to variability in image quality (i.e., cloud cover) and capture date, we were not able to use

images taken on identical dates across years. Images were processed using the ERDAS Imagine remote sensing image analysis software (Intergraph, Madison, AL, USA) to apply radiometric corrections that eliminated background noise while retaining temporal variance in vegetative reflectance [52]. We used ERDAS Imagine to calculate NDVI on a pixel-by-pixel basis for each image based on the ratio of red to near-infrared reflectance [53]. For each site, we fit our observed NDVI values to a linear model:

$$NDVI = Year + Date + Date^2 + Year * Date + Year * Date^2 + \varepsilon,$$

to estimate daily NDVI values where $\varepsilon \approx N(0, \sigma^2)$. This model provided a good fit to the data ($R^2 > 0.80$, $F_{1,1} > 4.50$, $P < 0.001$). We used predicted values to estimate mean and maximum NDVI values for May, June, July, and summer (as defined above). We also estimated the mean NDVI value at the date of hatching, 15 days before hatch, date of each survival observation, and 5 and 10 days prior to the date of each observation. Finally, because variability in NDVI was low due to sagebrush obscuring the phenological progression of forbs and grasses, we adjusted all NDVI values by subtracting out the year and site specific NDVI value on 1 May. This linear transformation effectively removed baseline site and year variation, thereby allowing our analysis to focus more directly on the effect of within-year plant phenology at a site.

Analysis

Missing chicks whose fate could not be determined were removed (i.e., right-censored) from the data set at the time of their last confirmed detection. Failure to locate chicks may have been the result of transmitter failure, the chick being removed from the study site by a predator, or long distance movements that exceeded the range of the transmitters. On a few rare occasions, chicks were found alive several weeks after going missing. The flexibility of our model allowed us to reintroduce these chicks back into the data set once rediscovered. Alternatively, we could have assumed that missing chicks were either dead or alive but our approach likely provides the most realistic estimate of chick survival because only chicks with known fate were allowed to influence daily survival rates [17].

We modeled sage-grouse chick daily survival rates from hatch to 42-days of age using the known-fate maximum likelihood estimator developed by Manly and Schmutz [54] and extended by Fondell et al. [55]. This model assumes a piecewise survival function such that the survival rate from age t to age $t+1$ is:

$$\Phi_t = \exp(-\alpha_t)$$

where $\alpha_i \geq 0$, for $t_{i-1} \leq t < t_i$, with $t_0 = 0$, and $i = 1, 2, \dots, p$. Therefore the daily survival rate (DSR) for ages 0 to t_1 days is assumed to be $\exp(-\alpha_1)$, the DSR for ages t_1 to t_2 days is assumed to be $\exp(-\alpha_2)$, and so forth, with p survival intervals. If N_a chicks are observed in a brood at age a then the number of survivors at age $b > a$, N_b , has a binomial distribution with mean:

$$E(N_b | N_a) = N_a \Phi_a \Phi_{a+1} \cdots \Phi_{b-1}.$$

To account for extra-binomial variance, the variance term is:

$$V_1(N_b | N_a) = D * V(N_b | N_a),$$

Where D is a constant and $V(N_b | N_a)$ is the binomial variance given by:

$$V(N_b | N_a) = N_a \Phi_a \Phi_{a+1} \cdots \Phi_{b-1} (1 - \Phi_a \Phi_{a+1} \cdots \Phi_{b-1}).$$

This variance formulation assumes that most extra-binomial variation is the result of lack of independence in the fates of chicks within broods. Given this formulation and assumptions, the log-likelihood function for the observed number n_b , at the end of a survival period is derived from the normal density function and takes the form:

$$L(\alpha_1, \alpha_2, \dots, \alpha_p, D) = \sum \left[-0.5 \log_e \{ 2\pi V_1(N_b | N_a) \} - \frac{0.5 \{ n_b - E(N_b | N_a) \}^2}{V_1(N_b | N_a)} \right],$$

where the summation is over all the instances in the data set where a brood size is observed at time a and then observed next at time b [54].

This generalized linear model is appropriate because it allows for variable observation intervals, changes in brood size due to missing chicks, and accounts for lack of independence in fates among chicks within a brood by using a quasi-likelihood approach [17,54,55]. Values of D near 1 indicate minimal dependence in the fates of brood mates whereas larger values correspond to decreasing independence among brood mates [54]. Covariates were modeled using a logit-link. Maximum likelihood estimates for all parameters were estimated using the 'OPTIM' function in R 2.14.1 [56].

To examine processes affecting chick survival in our populations, we first developed models that included alternative parameterizations of chick age. For example, we created models with categorical age classes wherein the categories were based on biological development of chicks, such as pre- versus post-flight ages or early ages when the diet consists primarily of insects versus later ages when forbs become important. We also considered linear and quadratic models of age treated as a continuous variable. Competing models of the various chick age parameterizations were ranked using the quasi-likelihood version of Akaike's Information Criterion adjusted for sample size (QAIC_c: [57,58]). Models with $\Delta QAIC_c \leq 2$ were considered to be equally supported by the data, and when this occurred we applied the principle of parsimony and based our inference on the model with the fewest parameters [59]. Upon identifying the best parameterization of chick age, we next considered the addition of hen age (SY or ASY) and hatch date effects, as both have been shown to be important predictors of sage-grouse chick survival [17,60]. Year and site effects were not modeled explicitly because all covariates of interest were site and year specific (i.e., site and year effects were modeled implicitly). The validity of the approach was assessed by adding year and site effects to our final top model and monitoring the change in QAIC_c.

We then developed candidate model sets for each of the 3 covariate groups. Covariates within each group tended to be correlated. To insure the interpretability of parameter estimates (i.e., to avoid multicollinearity), covariates with a Pearson correlation coefficient (ρ) greater than 0.50 were not included in the same model. To determine which of the 3 groups of covariates had the greatest impact on chick survival, we did not include covariates from different groups in the same model. These restrictions limited the complexity of models we considered. Upon identifying the top model for each of the 3 covariate groups, we

obtained 95% bootstrap confidence intervals for model parameters using 5,000 samples with replacement from our dataset [61]. All continuous covariates were Z-standardized prior to analyses. We calculated the proportional reduction in deviance [62] for each model relative to the null model:

$$D_I = 1 - (dev_I / dev_N)$$

where D_I is the Zheng-score for the model of interest, dev_I is the deviance for the model of interest, and dev_N is the deviance for the null model (unless otherwise noted, an intercept-only model) and deviance was calculated as $-2 \times \text{quasi-log-likelihood}$. The Zheng-score is a goodness-of-fit measure for generalized linear models of longitudinal data and can be interpreted similarly to a standard coefficient of determination, R^2 , in a linear model [62]. We then further assessed model fit by calculating the ratio of the Zheng-score for the model of interest relative to the spatially and temporally saturated model [63]:

$$R_I = D_I / D_{FS}$$

where D_I is the Zheng-score for the model of interest and D_{FS} is the Zheng-score for the fully spatial and temporally saturated model. Values of R close to zero indicate little improvement in model fit over the null model, whereas values of R that approach one indicate model fit similar to the fully saturated model.

Results

Chick statistics

Most hens had a single brood during the course of our multi-year study; however, 24 of the 142 hens had broods during more than one year of the study. Peak hatch date ranged from 25 May to 7 June at the Utah study area and from 19 May to 30 May for the Idaho area. During the 9 years of study we attached radio transmitters to 518 chicks from 142 broods, resulting in 11,188 chick exposure days (Table 1). Chick age at the time of capture ranged from 1 to 8 days. A total of 18 chicks were determined to have died as a result of capture, and were excluded from analyses.

Table 1. Capture statistics for greater sage-grouse chicks marked in Idaho (1999–2002) and Utah (2005–2009).

Year	Broods ¹	Chicks ²	Hen Ages ³	Marked ⁴
1999	13	30	SY = 3, ASY = 10	2.31
2000	15	42	SY = 4, ASY = 11	2.80
2001	14	40	SY = 1, ASY = 13	2.86
2002	24	71	SY = 5, ASY = 19	2.96
2005	21	89	SY = 11, ASY = 10	4.24
2006	21	61	SY = 0, ASY = 21	2.90
2007	12	55	SY = 4, ASY = 8	4.58
2008	11	66	SY = 2, ASY = 9	6.00
2009	11	64	SY = 1, ASY = 10	5.82
Total	142	518	SY = 31, ASY = 111	3.65

¹Number of broods captured.

²Total number of chicks marked with radio-transmitters.

³SY = second year hen (hatched the previous year), ASY = after second year hen (hatched ≥ 2 years earlier).

⁴Average number of chicks marked per brood.

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We censored an additional 159 missing chicks from the dataset after the last date of telemetry observation.

Base null model

Our best intra-annual model of chick survival included linear and quadratic effects of chick age (Table S1). This model clearly out-performed all other intra-annual temporal models in terms of QAICc value. This model was then used as the base model for evaluation of the main effects of hen age and hatch date. Comparison of QAICc scores for these models (Table S2) shows that the model including only hen age and the additive effects of hen age and hatch date were both competitive ($\Delta \text{QAICc} < 2$). Because the model containing only the effect of hen age was more parsimonious, we chose to retain this model as the base null model for comparison of climate, drought, and greenness phenology covariates [64].

NDVI models

All NDVI covariates considered were highly correlated (all $\rho > 0.62$). Thus, we did not construct models containing multiple NDVI covariates. All 13 single NDVI-effect models produced positive beta estimates (Table S3). Five models were equally supported by the data ($\Delta \text{QAICc} < 2.0$, Table S3). However, none of the 13 models, including the top 5 models, resulted in a meaningful increase in model fit (as measured by the R-score) relative to the base model. Additionally, the 95% confidence intervals for the effect of the average NDVI in July relative to May 1 for a given site and year (the model with lowest QAICc) were symmetrical around zero, indicating a weak and imprecise effect (Table S4).

Drought models

As with our NDVI covariates, all drought covariates were highly correlated (all $\rho > 0.77$) so only single-effect models were considered. Of the 10 drought models considered, the model including the effect of the PZI for the preceding winter performed best (Table S5). The addition of winter PZI to the base model resulted in an approximate 40% increase in the R-score, indicating a substantial improvement in model fit. Further, the 95% confidence interval for winter PZI indicated a significant positive effect of the covariate on chick survival (Fig. 2, Table S6).

Climate models

Several combinations of climatic covariates had correlation coefficients below our critical value. Additive effects of multiple climatic covariates were modeled if the correlation between all covariates was less than 0.5 and the model was deemed to be ecologically meaningful. These conditions resulted in the construction of 18 models (Table S7). The top climate model (minimum temperature in May+total precipitation in July) fit the data well (R-score = 0.766) and was the overall top model (Table 2). Both climatic effects in the top model were negatively associated with chick survival (Figs. 3–4). Despite the model fitting the data well, only the effect of July precipitation was significantly different from zero (Table S8). To ensure that the effects in our top model were robust and were not confounded by underlying effects of site and/or year, we added site and year effects to our top climate model (Table S9). Models containing year effects did not converge and models including additive and interactive site effects were not supported by the data (based on ΔQAICc), indicating that our results are robust across the 2 study sites. Allowing daily survival to change with chick age and holding all other covariate values at the sample mean, predicted values from our top model yielded a 42-

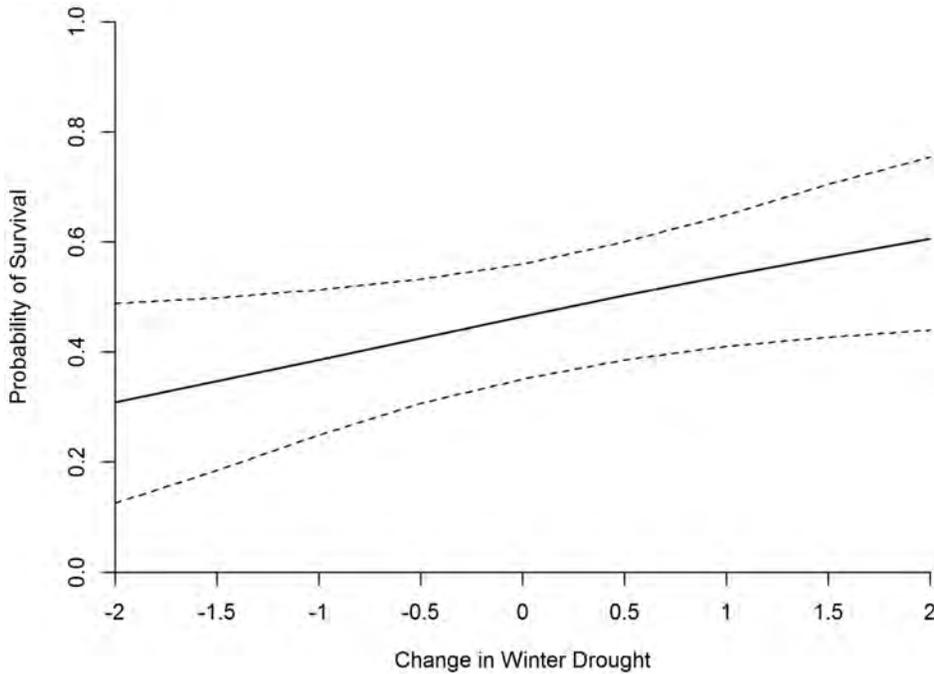


Figure 2. Effects of changes in winter drought severity (PZI) on the probability of greater sage-grouse chick survival to 42 days of age. Dashed lines indicate 95% confidence intervals. Negative values correspond to increasingly severe drought conditions. A change of 0.0 is equal to the mean Winter Palmer Z-Index score observed during the extent of this study. Palmer (1965) stated that a drought score of -2 was indicative of moderate drought.
doi:10.1371/journal.pone.0065582.g002

day survival probability of 0.475 (95% CI = 0.375 to 0.566). Estimates of *D* from the 3 top models all indicate that dependence in the fates among brood mates was low (1.6149 to 1.7085) but non-negligible (Tables S4, S6, S8).

Discussion

Studies of avian survival are often short-term and conducted on a single study area. While such studies provide important

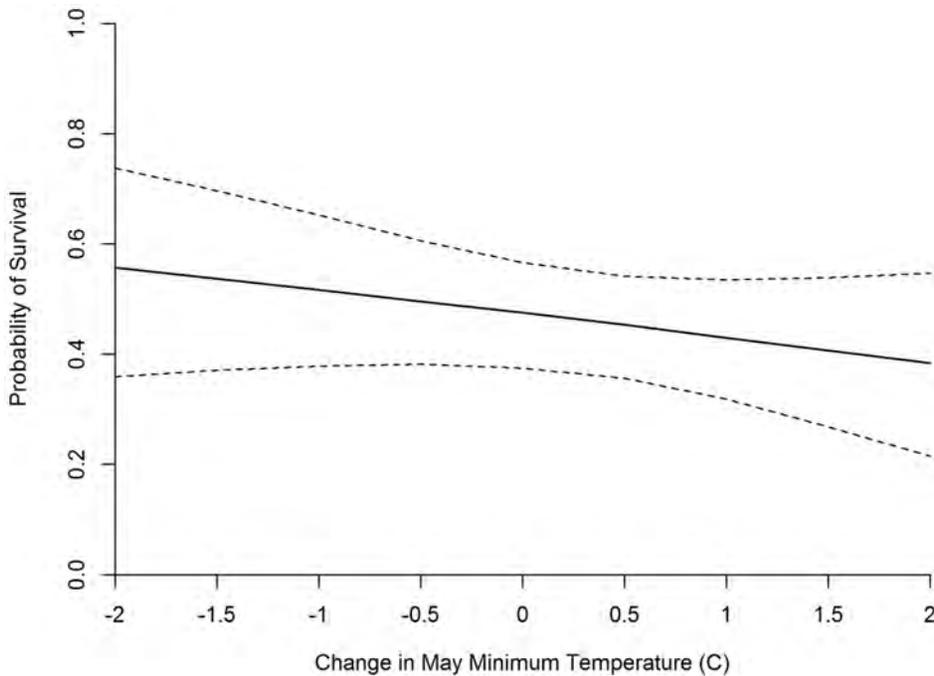


Figure 3. Effects of May minimum temperature on the probability of greater sage-grouse chick survival to 42 days of age. Dashed lines indicate 95% confidence intervals. A change of 0.0 is equal to the mean May minimum temperature observed during the extent of this study.
doi:10.1371/journal.pone.0065582.g003

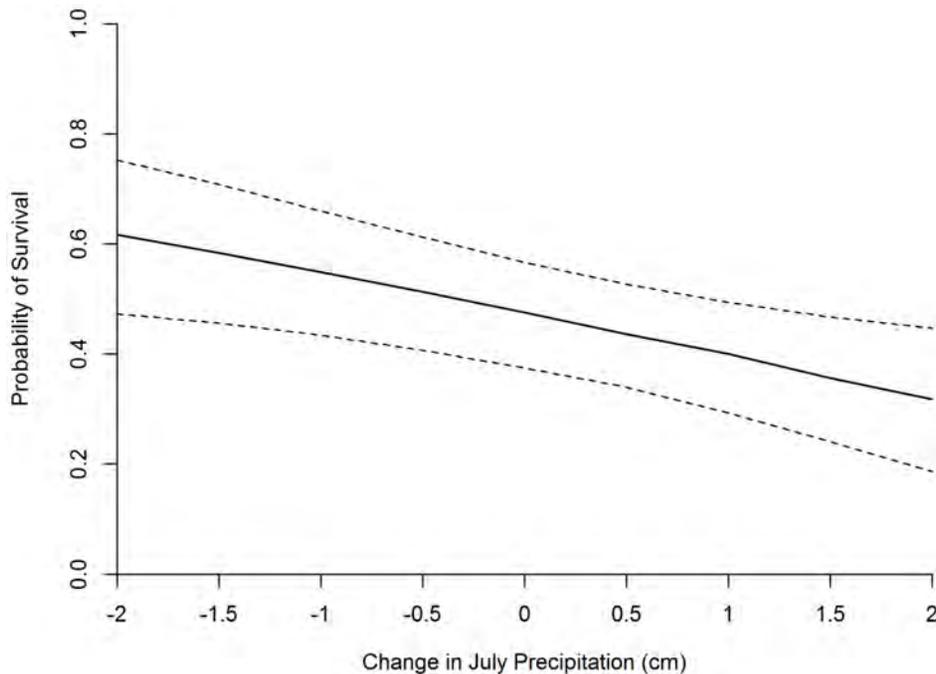


Figure 4. Effects of July precipitation on the probability of greater sage-grouse chick survival to 42 days of age. Dashed lines indicate 95% confidence intervals. A change of 0.0 is equal to the mean July precipitation observed during the extent of this study. doi:10.1371/journal.pone.0065582.g004

information, for many species there is a lack of knowledge concerning general large-scale factors which influence dynamics across space and time. An understanding of large-scale population drivers is essential for effective wildlife conservation planning and provides a baseline for developing meaningful hypotheses about specific local factors affecting populations at smaller spatial and temporal scales. Our study is the first to attempt to establish this

baseline for the survival of greater sage-grouse chicks across multiple populations.

Independence of brood mates

Our modeling approach allowed simultaneous incorporation of commonly collected demographic information (hatch date, chick age, hen age) as well as publically accessible landscape level biotic (NDVI) and abiotic (temperature, precipitation, drought) information into survival models implemented in R [56]. Additionally, our approach allowed us to account for the potential lack of independence among chicks from the same brood [55]. Estimates of D from the top 3 models (Tables S4, S6, S8) ranged from 1.6149 to 1.7085 and, in all cases, confidence intervals did not include one or the mean number of chicks marked per brood (3.65, Table 1). This finding indicates that, while not highly dependent, fates were not independent among brood mates. This supports our decision to use the Manly and Schmutz [54] survival estimator rather than traditional known-fate survival estimators that assume fates of individuals are independent.

Survival rate

Overall, chick survival during this study was relatively high. Our top model produced an average 42-day survival probability of 0.475 (95% CI = 0.375 to 0.566). This is similar to the 42-day survival rate of 0.50 reported for sage-grouse chicks by Dahlgren et al. [17] and considerably higher than the 28-day survival rate of 0.392 reported by Gregg and Crawford [18]. However, comparison of our observed survival rate to those of Gregg and Crawford [18] are potentially confounded by the use of different transmitter attachment methods (suture attachment versus subcutaneous implant). Gregg and Crawford [18] report a total of 32 chick mortalities attributable to capture compared to only 18 in our study despite a similar total number of chicks being marked in both studies. It is possible that our survival rates may be inflated if

Table 2. Comparison of top chick survival models among the landscape-scale covariate groups.

Model	K	QAICc	Δ QAICc	w_i	R-score
Base+Saturated Model	13	-121.45	0.00	0.999	1.000
Base+May Min Temp+July Precip (-,-)	7	-58.30	63.15	0.000	0.766
Base+Winter PZI (+)	6	58.21	179.66	0.000	0.396
Base+July Mean NDVI (+)	6	178.53	299.98	0.000	0.022
Quadratic Chick Age+Hen Age (Base)	5	183.48	304.93	0.000	0.000

All models contain the base effects of quadratic chick age and hen age. Models were evaluated using the Quasi-Akaike's Information Criterion (QAIC). K = number of parameters. w_i = model weight (i.e. the likelihood of a particular model being the best model). R-score = percent reduction of deviance relative to the base model (Quadratic Chick Age+Hen Age). The saturated model contains effects for site (1 parameter) and each year (7 parameters). Typically 8 parameters would be required to model the effects of 9 years. However, because years did not overlap between the 2 sites we were able to fully specify year effects with only 7 parameters. doi:10.1371/journal.pone.0065582.t002

some chicks treated as capture mortalities were incorrectly classified as such. However, the low incidence of chicks being classified as capture mortalities makes it unlikely that any misclassifications would significantly influence our findings.

Our analysis supported previous research that has shown both chick age and hen age to be important predictors of sage-grouse chick survival [17,60] (Tables S1 and S2). Interestingly, our models indicate that chicks hatched to second-year hens experience higher survival rates than chicks hatched to older hens. This effect has been previously reported for sage-grouse [17,60] although the mechanism underlying it has not been thoroughly explained. Despite being poorly estimated throughout (Tables S4, S6, S8), we chose to retain chick and hen age covariates in all models to minimize bias in estimates of the effects of interest.

Effects of NDVI

In recent years, NDVI has proven to be a useful tool for understanding various aspects of animal ecology [65]. We found positive relationships between all of our measures of NDVI phenology and chick survival (Table S3). However, none of the NDVI measures resulted in substantial improvements in model fit, as measured by the R-score, relative to the base (chick age+hen age) model, nor were the effects significant. Blomberg et al. [27] similarly found that NDVI was positively associated with sage-grouse recruitment and population growth, but that NDVI provided weak predictive power relative to other predictors such as precipitation.

Given the importance of invertebrates and herbaceous vegetation in the diet of sage-grouse chicks [23,18,25], the poor predictive power of NDVI for sage-grouse chick survival is somewhat surprising because NDVI is a well-established index of net primary production [65,66,67], and invertebrate production is positively related to plant production [68]. We suggest that the extensive coverage of sagebrush across both study sites resulted in phenological measures of NDVI being less sensitive to changes in coverage of forbs and grasses, thereby diminishing the ability of NDVI to measure changes of direct relevance to sage-grouse chicks. Correspondingly, Paruelo and Lauenroth [29] found a generally smaller range of NDVI values in sagebrush-steppe ecosystems than in grasslands where plant phenological changes are likely easier to detect.

Effects of drought

Although local availability and abundance of specific invertebrates and forbs is proximally related to sage-grouse chick survival [17,18], survival is likely under the primary influence of physical factors such as precipitation (amount and timing), temperature, and drought. Accordingly, our analysis indicated that abiotic factors were better predictors of sage-grouse chick survival than phenology of NDVI. Our top drought model (Table S5) indicated the presence of a significant relationship between winter drought and chick survival (Table S6). Since smaller PZI (and PDSI) values correspond to increasingly severe drought conditions, the positive parameter estimate associated with the winter drought effect implies that winter droughts lead to reduced chick survival (Fig. 2). Unfortunately, our data do not allow us to identify the true causal mechanism(s) underlying this relationship. Schwinning et al. [69] found that winter drought, even more so than summer drought, affects plant production during the following summer. Therefore, winter drought may affect sage-grouse chick survival via its influence on brood habitat quality. Additionally, winter drought may influence chick survival by affecting resource provisioning during egg formation. Forb abundance during the pre-nesting period is positively associated with hen nutrition [70], and hen

nutrition prior to nest initiation is positively related to reproductive investment [71]. Thus, we suggest that either or both of these effects may be the mechanism behind the relationship between winter drought and chick survival that we observed.

Effects of climate

Blomberg et al. [27] reported relatively stable survival rates for adult sage-grouse but found that recruitment was variable and strongly influenced by annual climatic variation. These findings led the authors to conclude that stability of sage-grouse populations is dependent upon stable annual survival rates and occasional large inputs of new individuals into the population when climatic conditions are amenable to chick and juvenile survival. Our results support this assertion that climatic variables play a primary role in determining sage-grouse reproductive success. Of the 3 groups of predictors of chick survival we considered, models containing climatic effects clearly outperformed all other models (Table S7 versus Tables S5 and S3).

Our top climatic model fit the data well (Table 2, R-score = 0.766). In addition to the effects of chick and hen age, the top model included the minimum temperature in May (MMT) and precipitation in July, both producing negative parameter estimates (Table S8). We initially hypothesized that MMT could have either a positive or negative association with chick survival. Specifically, we predicted that MMT could be positive if higher minimum temperature resulted in fewer chicks dying due to exposure. Alternatively, we predicted that a negative effect of MMT would be attributable to high minimum temperatures leading to early snow melt and thus lower soil moisture and poor habitat quality throughout the brood-rearing period. We conclude that the latter is the case. Although particularly cold temperatures in late May could potentially result in increased exposure mortality, consideration of our peak hatch dates (see Section 3.1) reveals that it is unlikely that many chicks would be hatched early enough to be exposed to extreme low temperatures likely occurring in the first half of May. We also note that the minimum temperature in June is positively associated with chick survival, possibly indicating that exposure mortality does increase as temperature decreases during this timeframe.

Our interpretation of the negative effect of MMT on chick survival does raise concerns about the impact of projected climate on future sage-grouse reproductive success. Significant temperature increases have been documented across western North America in recent decades, and climate models consistently predict that temperatures will continue to increase into the foreseeable future [72]. Observed and projected warming trends have also been connected to observed and projected transitions from winter precipitation falling as rain rather than snow and consequently reduced spring snow pack [72,73,74].

The trend in warming temperatures could impact sage-grouse population dynamics as a result of phenological asynchrony [75], increased spread of exotic species such as red fox (*Vulpes vulpes*; [76]) and cheatgrass (*Bromus tectorum*; [5]), and increased frequency and severity of wildfires [77]. Blomberg et al. [27] concluded that projected climate change could result in reduced recruitment of sage-grouse. Our results support this conclusion. Figure 3 shows model-derived chick survival estimates across a range of MMT. According to our results, a 2°C increase in mean MMT, well within the range projected by most climate models [72], will result in an approximate 10% reduction in sage-grouse chick survival. This effect could be mitigated if sage-grouse are able to adjust their hatch dates to correspond with earlier snow melt and advanced plant phenology. A simple linear regression of our observed median hatch dates on MMT shows a significant correlation

($p = 0.0171$, $R^2 = 0.58$). This demonstrates that sage-grouse may be capable of synchronizing the timing of nesting with MMT for at least the range of MMT observed during our study. However, it is not clear if the level of plasticity in breeding phenology is sufficient to compensate for future climatic changes. Additionally, if warming results in a shift in the form of winter precipitation from snow to rain [73], chick survival may still be negatively affected by poor habitat quality, even if hens are able to adjust nest initiation to correspond with early snowmelt.

We initially hypothesized that July precipitation (JP) would have a positive effect on sage-grouse chick survival due to a moisture associated increase in plant and arthropod forage. However, our analysis showed a significant negative effect of JP (Table S8). While this result may be less intuitive, we conclude that it is real and meaningful. At least 2 mechanisms may underlie the relationship between chick survival and JP. First, chicks may be susceptible to exposure mortality in July. As noted above, the Utah study area is located in a monsoonal zone and receives a substantial proportion of the annual precipitation during late summer (primarily July and August). Monsoonal storms across the Utah study area often build quickly and result in significant temperature reductions followed by rain, hail, or both. By July, chicks are larger in size and are more independent of the brood hen [78]. If chicks are too large to be effectively brooded, severe monsoonal storms may result in chicks becoming soaked by rain and/or losing body temperature due to low temperatures or hail. While the Idaho study area is not in a monsoonal zone, it is possible that occasional severe July storms could produce similar effects. However, by July chicks should be more capable of thermoregulation relative to the early development period in June. Thus, we conclude that if exposure were a major source of chick mortality, models including the effect of June precipitation or the minimum temperature in June would have performed better.

Alternatively, JP may negatively affect sage-grouse chick survival through an interaction between increased moisture/humidity and predator search efficiency (i.e., moisture facilitated predation hypothesis). Moisture on skin and feathers increases bacterial activity, subsequently increasing scent production [36,37,38,39,40]. Mammalian predators have been shown to respond rapidly to the presence of prey odor [79] and increased scent production may lead to enhanced prey detection rates. A number of studies have found increased nest predation rates following precipitation events for greater sage-grouse and other gallinaceous birds [33,80,81], and the phenomenon of moisture-facilitated predation may apply to chicks and adult birds as well [34,82]. We do not present observed chick predation rates due to concerns about correctly distinguishing between predation and scavenging. However, both of our study sites were inhabited by a suite of potential mammalian predators. Both study sites received predator management to reduce coyote (*Canis latrans*) predation on livestock, but coyotes and other common predators of sage-grouse chicks (red fox, badger [*Taxidea taxus*], weasels [*Mustella* sp.], and rattlesnakes [*Crotalus viridis*]) were present on both sites. In addition to the potential effects of moisture-facilitated predation by olfactory predators, JP may increase predation by avian predators if sage-grouse broods move to areas with less sagebrush cover following precipitation events to expedite drying and/or warming. Although not formally documented, we did observe broods along roadways at a higher frequency following precipitation events than at other times.

Effects of climate change on precipitation are less clear than the effects on temperature [72]. Climate models are inconclusive as to the sign of the effect on precipitation [83], and effects may vary by season [84]. In the absence of a consensus about effects of climate

change on summer precipitation, anticipating the effect of changing precipitation on ecological communities and populations is difficult. Our analysis indicates that a 2 cm change in JP (positive or negative) would result in an approximate 15% change in sage-grouse chick survival (Fig. 4).

Sage-grouse are a species of great conservation concern in western North America. Chick survival has been shown to be an important determinant of population growth rates [9,10], yet relatively little is known about climatic or other large-scale environmental factors affecting survival rates. Previous studies have identified specific habitat characteristics that influence survival [17,18]. These studies have led to a proliferation of efforts to manage brood-rearing habitats without thorough consideration of the abiotic factors influencing both habitat quality and chick survival. Our study clearly demonstrates that large-scale abiotic factors such as drought, temperature and precipitation have significant effects on chick survival. These factors are beyond the control of state and federal wildlife management agencies and highlight the importance of considering current and future climatic conditions when developing policy and conservation strategies for this species. However, the effects we observed were measured for populations inhabiting large intact tracts of sagebrush habitat. The availability of adequate amounts of suitable habitat is a prerequisite that must be met for the effects of the abiotic factors we studied to be relevant.

Supporting Information

Table S1 Models for effect of age on greater sage-grouse chick survival.

(DOCX)

Table S2 Models for effect of hen age and hatch date on greater sage-grouse chick survival.

'Age' is the top age varying model from Table S1. Signs in parentheses indicate the direction of respective covariate effects excluding chick age.

(DOCX)

Table S3 Models for the effects of habitat greenness as measured by the Normalized Difference Vegetation Index (NDVI) on greater sage-grouse chick survival.

Signs in parentheses indicate the direction of respective covariate effects excluding chick age. All models (except the intercept only model) contain the base effects of quadratic chick age and hen age. Models were evaluated using the Quasi-Akaike's Information Criterion (QAIC). K = number of parameters. w_i = model weight (i.e. the likelihood of a particular model being the best model). R-score = percent reduction of deviance relative to the base model (Quadratic Chick Age+Hen Age).

(DOCX)

Table S4 Parameter estimates with 95% confidence intervals for the top model of the effects of Normalized Difference Vegetation Index (NDVI) on greater sage-grouse chick survival.

Confidence intervals were calculated based on 5,000 bootstraps of the original data set.

(DOCX)

Table S5 Models for the effects of drought on greater sage-grouse chick survival.

Signs in parentheses indicate the direction of respective covariate effects excluding chick age. All models (except the intercept only model) contain the base effects of quadratic chick age and hen age. Models were evaluated using the Quasi-Akaike's Information Criterion (QAIC). K = number of parameters. w_i = model weight (i.e. the likelihood of a particular model being the best model). R-score = percent reduction of

deviance relative to the base model (Quadratic Chick Age+Hen Age). (DOCX)

Table S6 Parameter estimates with 95% confidence intervals for the top model of the effect of drought on greater sage-grouse chick survival. Confidence intervals were calculated based on 5,000 bootstraps of the original data set. (DOCX)

Table S7 Models for the effects of climate on greater sage-grouse chick survival. Signs in parentheses indicate the direction of respective covariate effects excluding chick age. All models (except the intercept only model) contain the base effects of quadratic chick age and hen age. Models were evaluated using the Quasi-Akaike's Information Criterion (QAIC). K = number of parameters. w_i = model weight (i.e. the likelihood of a particular model being the best model). R-score = percent reduction of deviance relative to the base model (Quadratic Chick Age+Hen Age). (DOCX)

Table S8 Parameter estimates with 95% confidence intervals for the top model of the effects of climate on

greater sage-grouse chick survival. Confidence intervals were calculated based on 5,000 bootstraps of the original data set. (DOCX)

Table S9 Evaluation of the effects of site and year effects on the top model from Table S7. All models contain the base effects of quadratic chick age and hen age. Models were evaluated using the Quasi-Akaike's Information Criterion (QAIC). K = number of parameters. w_i = model weight (i.e. the likelihood of a particular model being the best model). (DOCX)

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Author Contributions

Conceived and designed the experiments: MRG DKD TAM JWC KPR NB. Performed the experiments: MRG DKD NB. Analyzed the data: MRG PAT DNK. Wrote the paper: MRG.

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Tools and Technology

Mapping Sage-Grouse Fence-Collision Risk: Spatially Explicit Models for Targeting Conservation Implementation

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ABSTRACT Recent research suggested greater sage-grouse (*Centrocercus urophasianus*; hereafter, sage-grouse) fence collision may be widespread, and fence-marking methods have been developed for reducing prairie-grouse collision in sagebrush-steppe habitats. However, research also suggested sage-grouse collision was highly variable, and managers implementing mitigation desire targeting tools to prioritize mitigation efforts as a function of risk. We fit collision-risk models using widely available covariates to a sage-grouse fence-collision data set from Idaho, USA, and developed spatially explicit versions of the top model for all known sage-grouse breeding habitats (i.e., within 3 km of leks) in 10 of 11 western states where sage-grouse are found. Our models prioritize breeding habitats for mitigation as a function of terrain ruggedness and distance to nearest lek, and suggest that a relatively small proportion of the total landscape (6–14%) in each state would result in >1 collision over a lekking season. Managers can use resulting models to prioritize fence-marking by focusing efforts on high risk landscapes. Moreover, our models provide a spatially explicit tool to efficiently target conservation investments, and exemplify the way that researchers and managers can work together to turn scientific understanding into effective conservation solutions. © 2013 The Wildlife Society.

KEY WORDS avian collision, *Centrocercus urophasianus*, collision mitigation, fence collision, fence markers, infrastructure marking, sage-grouse.

Collision with elevated structures is a common phenomenon for many species of grouse (Catt et al. 1994, Baines and Summers 1997, Wolfe et al. 2007, Stevens et al. 2012a). Early research from Europe reported grouse among the most common infrastructure-collision victims, and suggested tetraonid collision susceptibility may be a function of morphology (e.g., heavy body wt, high wing loading; Baines and Summers 1997, Bevanger 1998, Bevanger and Brøseth 2000, Janss 2000). More recently, research in North America suggested prairie-grouse are susceptible to collision with fences (Patten et al. 2005, Wolfe et al. 2007, Stevens et al. 2012a). Fence collision was attributed to 39.8% of mortality for lesser prairie chickens (*Tympanuchus pallidicinctus*) in Oklahoma, USA (Wolfe et al. 2007), and uncorrected mean fence-collision rates of 0.38–0.41 strikes/

km were reported for greater sage-grouse (*Centrocercus urophasianus*; hereafter, sage-grouse) during the breeding season in Idaho, USA (Stevens 2011). Fences and other anthropogenic structures are ubiquitous across western North America (Braun 1998, Knick et al. 2011); however, population-level impacts of prairie-grouse collision are poorly understood.

Infrastructure marking is a commonly suggested conservation strategy for reducing avian–infrastructure collision (Baines and Andrew 2003, Wolfe et al. 2009, Stevens et al. 2012b). Power-line markers appear to reduce collision for a variety of avian species (Morkill and Anderson 1991, Brown and Drewien 1995, Savereno et al. 1996, Barrientos et al. 2011), but assessments of fence-markers are less common. However, orange barrier netting reduced woodland grouse fence-collision in Scotland (Baines and Andrew 2003). Moreover, fence-marking methods have been developed for North American prairie grouse (Wolfe et al. 2009; Fig. 1), and evidence from Idaho suggested marking reduced the count of sage-grouse collisions by 83% during the breeding season (Stevens et al. 2012b).

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Figure 1. Male greater sage-grouse displaying on a lek directly beside a marked fence on an Idaho, USA, study site. Reflective fence markers were shown to reduce sage-grouse collision counts by approximately 83% in high-risk breeding habitats (Stevens et al. 2012b).

Managers are forced to make decisions with incomplete information and constrained budgets, and efficient allocation of resources promotes the greatest return on conservation investments (Bottrill et al. 2008). Targeting conservation to ensure that funds are allocated efficiently is often referred to as triage, a process that provides transparency and forces managers to consider opportunity costs of management actions (Bottrill et al. 2008). Sage-grouse collision appears highly variable within and between regions (Stevens et al. 2012a, b). Variation in collision risk suggests mitigation is unnecessary at many sites and prioritizing mitigation as a function of risk may enable cost-effective implementation of mitigation efforts (Stevens et al. 2012a, b). Thus, small but targeted investments could potentially alleviate much of the fence-collision risk in breeding habitats, freeing up resources for other conservation efforts.

The science behind conservation planning is often not conducted in partnership with managers, further complicating management decisions and resource allocation. Instead, researchers often conduct studies with little input from end users and hope the conservation community finds it useful (Knight et al. 2008). Steps to alleviate this research-implementation gap include sourcing research questions directly from managers, fostering relationships between researchers and managers, and linking research to implementation of conservation actions. Research showing that fence marking can reduce sage-grouse collisions (Stevens et al. 2012b) has spurred fence-marking efforts on public and private lands across 11 western states. However, sage-grouse occupy vast areas of western North America (Schroeder et al. 2004), and wildlife managers desire spatially explicit targeting tools to maximize their return on conservation investments. Therefore, the objective of this study was to bridge the research-implementation gap by developing spatially explicit fence-collision-risk models for sage-grouse in breeding areas across the western United States. Specifically, we developed models by re-analyzing landscape

factors influencing collision risk from Stevens et al. (2012a), and applied resulting models to spatially predict and map fence-collision risk for all known sage-grouse breeding habitats in 10 of 11 western states.

STUDY AREA

We developed raster-regression models for areas within 3 km of all known and active sage-grouse leks ($n = 4,684$) in 10 of 11 states currently supporting sage-grouse. We used the most recently developed range-wide lek database for this analysis. The database was originally developed by Connelly et al. (2004), but has since been updated to reflect lek locations discovered and leks lost from 2004 to 2007 (Garton et al. 2011, Knick and Hanser 2011). Therefore, our analyses included all known and active sage-grouse leks as of 2007, although two states (ID and NV) provided lek location data updated through 2011.

METHODS

Stevens (2011) described a cluster sampling design used to survey fences in sage-grouse breeding areas of southern Idaho (2009: $n = 16$ sites; 2010: $n = 14$ sites), where 1×1 -km sampling units were randomly selected and surveyed during the breeding season at each site (Mar–May; 2009: $n = 60$ clusters; 2010: $n = 80$ clusters). The number of sage-grouse collisions per square km was recorded for each sampled cluster, and clusters were sampled on >1 occasion when possible, resulting in 224 collision-count observations (Stevens 2011). Stevens et al. (2012a) modeled these collision counts as a function of covariates, including distance from each 1×1 -km cluster's centroid to the nearest active lek, lek size (i.e., max. count) at the nearest lek, and a terrain ruggedness index (TRI; Riley et al. 1999). However, Stevens et al. (2012a) did not account for potential bias caused by removal of collision remains by scavengers, and only used a subset of collision-count observations representing the first sampling event at each site ($n = 123$). Therefore, we extended the analyses of Stevens et al. (2012a) and 1) used all 224 collision-count observations, 2) incorporated field-experiment data used to measure removal of collision evidence by scavengers, 3) used newly developed statistical models to combine collision-count data with removal-experiment data using joint-likelihood principles to estimate collision and removal process parameters, and 4) developed spatially explicit raster models to extrapolate estimated collision risk to all known sage-grouse breeding areas in 10 of 11 currently occupied states.

We modeled sage-grouse fence-collision counts from Idaho as a function of lek size, distance to lek, and TRI using a stochastic-process model for collision-count data developed by Stevens and Dennis (2013). Stevens et al. (2011) showed that removal of collision evidence prior to fence-collision sampling (i.e., evidence-removal bias) can be large, and removal of collision remains varied across regions of southern Idaho. The model used for our analyses predicts collision-count data with a generalized-regression approach that accounts for removal of collision evidence and accommodates covariates on collision- and removal-process

parameters (Stevens and Dennis 2013). The model treats instantaneous collision counts as a stochastic-linear-immigration-death (SLID) process (Matis and Kiffe 2000), whereby Poisson arrivals represent addition of collisions to the system (immigration) and proportional deaths remove evidence from a site. The SLID model combines collision-count and removal-experiment data sets to estimate collision (θ) and removal (ψ) rate parameters using joint likelihood. Stevens and Dennis (2013) showed that regional variation in evidence removal can result in order-of-magnitude differences in expected collision counts between regions with identical collision rates. Thus, the removal rate (ψ) is, in effect, a nuisance parameter, and failing to account for evidence removal when modeling avian-collision counts results in parameter estimates that are difficult to interpret (Stevens and Dennis 2013).

We combined data from collision-count surveys (Stevens 2011) with carcass-removal-experiment data (Stevens et al. 2011) to estimate parameters of the SLID model. We fit 14 total models and compared models using Akaike's Information Criterion (hereafter, AIC; Akaike 1973). We fit models using the log link function and seven different covariate combinations, where collision (θ) was modeled as a function of distance to lek, lek size, and TRI, and removal (ψ) was modeled as a function of a binary variable indicating study region (i.e., region of ID where removal experiments were conducted; 1 = southeast Idaho, 0 = Magic Valley region). For the region-specific removal, fences west of Craters of the Moon National Monument were considered the Magic Valley, whereas fences east of this location were located in southeast Idaho. We fit each of the seven covariate combinations using the transient and stationary versions of the model, by numerically maximizing the transition (i.e., time dependent) and stationary (i.e., equilibrium and time-independent) distribution joint likelihoods (Stevens and Dennis 2013). We generated profile-likelihood confidence intervals for all model parameters and conducted goodness-of-fit testing for the most supported model (Stevens and Dennis 2013). We used leave-one-out cross-validation and root-mean-squared error to evaluate prediction success, calculating square root of the average squared error between predicted and observed collision counts for each model. We used the R statistical computing language

for all model fitting and analyses (R Core Development Team 2006).

We developed spatially explicit models to predict collision as a function of covariates from the top SLID model. Because fence sampling in Idaho focused on areas within approximately 3 km of leks, we buffered all range-wide lek locations by 3 km in a Geographic Information System (GIS; ArcMap 10.0) and focused spatial analyses in these areas. We downloaded U.S. Geological Survey 30-m digital elevation models for each state (www.seamless.usgs.gov; accessed 7–9 Feb 2012), and calculated TRI for each 30-m pixel using ArcInfo. We calculated distance from each 30-m pixel to the nearest sage-grouse lek in GIS using the Euclidean distance function. Lastly, we used the raster calculator in GIS to extrapolate maximum-likelihood estimates of the total number of sage-grouse collisions over a lekking season for each 30-m pixel as a function of distance to lek and TRI, assuming a 78-day lekking season (15 March to 31 May; $\hat{y} = 78 \times \exp(\beta_0 + \beta_1 \times \text{TRI} + \beta_2 \times \text{distance})$). The SLID model explicitly accounts for evidence-removal bias in collision-count data, but does not account for detection error. Thus, our spatially-explicit models portray relative collision risk rather than absolute risk. Moreover, the predicted number of collisions for each 30-m pixel is entirely dependent on fence presence; obviously, not all pixels across the landscape have fences present. Lastly, we used an example collision-risk threshold of >1 collision/lekking season, and calculated the proportion of the 30-m pixels with a collision risk above this value for each state.

RESULTS

Modeling identified TRI and distance to lek effects on collision rates, and regional differences in removal of collision evidence ($\Delta\text{AIC} = 0$; Table 1). The top model suggested collision decreased with increasing TRI ($\beta = -0.25$; 95% CI = -0.48 to -0.10 ; Fig. 2) and increasing distance from the nearest sage-grouse lek ($\beta = -0.0006$; 95% CI = -0.00115 to -0.00008 ; Fig. 2). Thus, an increase in topographic variation at a site and moving farther from a lek location strongly reduced the number of collisions predicted over a lekking season (Fig. 2), and sites predicted to be high risk were concentrated on flat areas in relatively close proximity to leks (Fig. 3). Goodness-of-fit testing failed to

Table 1. Model rankings for the stochastic linear-immigration-death model fit to the greater sage-grouse fence-collision data set from southern Idaho, USA. Covariates were size of nearest lek (lsize), distance to nearest lek (dist), terrain ruggedness index (TRI), and region (SE ID = 1, Magic Valley = 0; Stevens et al. 2011). Models were ranked and compared using Akaike's Information Criterion (AIC; Akaike 1973).

Model ^{a,b}	K^c	ΔAIC	AIC
$\theta(\text{TRI} + \text{distance}) \psi(\text{region})$	5	0	403.505
$\theta(\text{TRI} + \text{lsize} + \text{distance}) \psi(\text{region})$	6	1.582	405.086
$\theta(\text{TRI}) \psi(\text{region})$	4	3.153	406.658
$\theta(\text{TRI} + \text{lsize}) \psi(\text{region})$	5	4.581	408.086
$\theta(\text{distance}) \psi(\text{region})$	4	12.210	415.715

^a Model form is $\log(\theta) = \beta_0 + \beta_1 Y_1 + \dots + \beta_k Y_k$ and $\log(\psi) = \gamma_0 + \gamma_1 Y_1 + \dots + \gamma_k Y_k$, where θ = daily collision rate and ψ = per capita daily removal rate (Stevens and Dennis 2013).

^b All top models were fit using the transient joint likelihood for collision-count observations after the first sampling occasion (Stevens and Dennis 2013). No models fit using the stationary joint likelihood for all count observations were supported by the data ($\Delta\text{AIC} > 19$).

^c K = no. of model parameters.

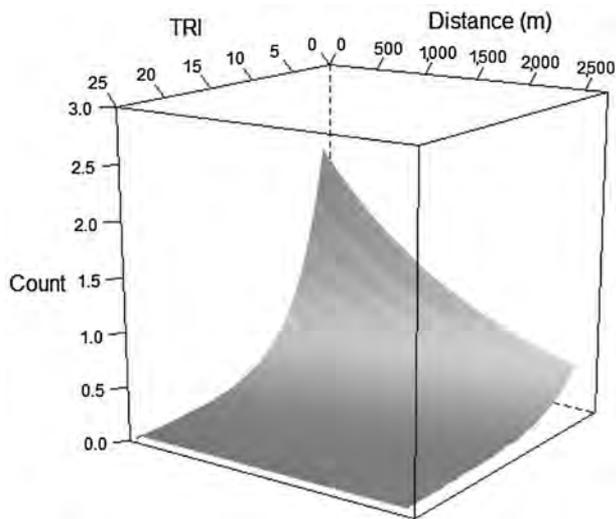


Figure 2. Maximum-likelihood estimates of total number of greater sage-grouse fence collisions over the 78-day lekking season from the top stochastic-linear-immigration-death model fit to data from southern Idaho, USA. Collision was a function of terrain ruggedness (TRI) and distance to the nearest lek. Maximum-likelihood estimates of total collisions from the top model = $78 \times \exp\{\beta_0 + \beta_1 \times \text{TRI} + \beta_2 \times \text{distance}\}$.

reject the hypothesis that the top model fit the data ($P = 0.16$, $\chi^2_{249} = 271.22$), and cross-validated prediction error was similar among top three models (range = 0.634–0.648). The raster regression models demonstrated the large variability of predicted collisions per 30-m pixel across the landscape, and suggested that a relatively small proportion of the total landscape (6–14%) in each state would result in >1 collision over a lekking season (Fig. 3; Table 2). Despite spatial variation in collision risk, Idaho, South Dakota, California, Montana, and Oregon all had >10% of their area within 3 km of active leks with >1 predicted collision over a

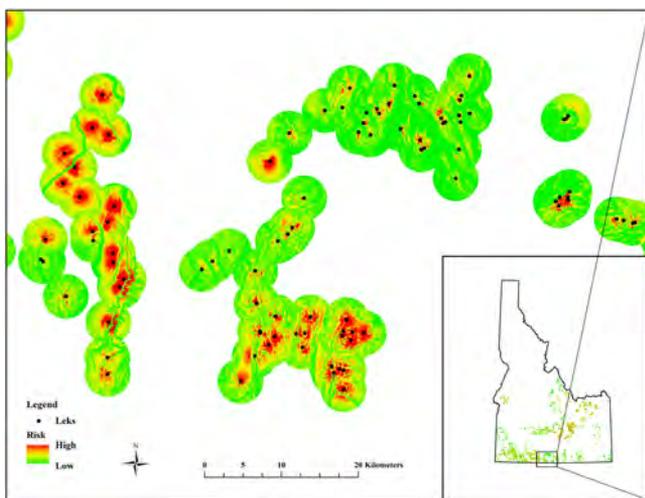


Figure 3. Example of spatially explicit fence-collision-risk maps from greater sage-grouse breeding habitats of southern Idaho, USA. Collision risk was a function of terrain ruggedness (TRI) and distance to the nearest lek. Maximum-likelihood estimates of total collisions (i.e., risk) from the top stochastic-linear-immigration-death model = $78 \times \exp\{\beta_0 + \beta_1 \times \text{TRI} + \beta_2 \times \text{distance}\}$.

lekking season (Table 2). Montana (465,631 ha), Wyoming (295,770 ha), and Idaho (214,184 ha) had the greatest total area with >1 predicted collision over a lekking season (Table 2). In contrast, Utah (6.3%), North Dakota (7.3%), and Washington (7.5%) had the lowest percentage of pixels within 3 km of leks with >1 predicted collision over a lekking season due to increased terrain ruggedness near lek locations (Table 2).

DISCUSSION

We created spatially explicit decision-support tools for wildlife and habitat managers who are marking fences to reduce sage-grouse collisions. Many previous avian-collision studies focused on known high-risk sites or used convenience-sampling methods to measure collision frequency, limiting generality of results and inferences. Moreover, rapid removal of collision remains can decrease accuracy of collision counts and bias estimates of collision totals (Smallwood 2007, Huso 2011, Stevens et al. 2011). We attempted to avoid pitfalls in study design by randomly sampling fences from sites spread across southern Idaho ($n = 14\text{--}16$ sites; Stevens et al. 2012a), measuring evidence removal with field experimentation (Stevens et al. 2011), and combining these data sets to model collision (θ) and removal (ψ) as a function of covariates using joint likelihood and generalized regression (Table 1). The models identified terrain ruggedness and distance from the lek metrics as drivers of fence-collision risk (Fig. 2; Stevens et al. 2012a). We hypothesize that collision risk is ultimately influenced by grouse flight behavior in flat terrain, where grouse fly low into leks before dawn and are thus vulnerable to colliding with fences. We found some evidence for the effect of lek size on collision ($\Delta\text{AIC} = 1.5$; Table 1). However, our analyses suggested topography and distance were better predictors of collision than counts of displaying males on leks. This does not necessarily mean that local abundance does not influence collision risk, and measurement error in lek count indices may have attenuated the estimated effect on collision. Moreover, other covariates influencing sage-grouse collision were intentionally excluded from our analyses because they were not available at the range-wide extent (e.g., fence density; Stevens et al. 2012a). Regardless, terrain ruggedness attenuated other covariate effects and drove collision risk to nearly zero at moderate–high values (Fig. 2).

This study bridges the research-implementation gap by working in partnership with managers implementing mitigation measures to design user-friendly maps that suggest where targeted investments could alleviate much of the breeding season collision risk, freeing up resources for more pressing conservation concerns (Knight et al. 2008, Black and Groombridge 2010). Our models suggest that most of the breeding-area landscape across the West has low collision risk. As such, these models facilitate appropriate regional-scale resource allocation, by suggesting that targeted marking efforts may be beneficial to sage-grouse but that marking efforts are not necessary near all leks. We developed these maps at broad scales using covariate data that are widely available (e.g., terrain ruggedness); additional

Table 2. Summary statistics from spatially explicit fence-collision models in sage-grouse breeding habitats across the western United States. Statistics are: mean and standard deviation (SD) of predicted collision count per 30-m pixel, percent of the landscape (i.e., percent of total pixels) with >1 predicted collision over the lekking season (% >1 collision), and the number of hectares within 3 km of known leks (i.e., no. of pixels \times 0.09 ha/pixel) with >1 predicted collision over the lekking season for each state. Both the percent of landscape and total area (ha) with >1 predicted collision over the lekking season are predicated on the presence of fence in each 30-m pixel.

State	\bar{x}	SD	% > 1 collision ^a	Area (ha) > 1 collision
ID	0.509	0.472	14.413	214,184
SD	0.563	0.413	13.107	6,933
CA	0.426	0.450	11.381	15,303
MT	0.477	0.415	11.157	465,631
OR	0.435	0.436	10.886	91,305
WY	0.422	0.403	9.239	295,770
NV	0.393	0.399	8.544	107,758
WA	0.397	0.375	7.531	4,715
ND	0.394	0.376	7.330	3,964
UT	0.319	0.369	6.264	28,380

^a Max. of the predicted no. of collisions per 30-m pixel over a breeding season = 3.027 birds.

information at local scales (e.g., fence locations or densities, local space use) can be used to further inform management actions. Thus, our models can be used for local-scale planning by managers working in conjunction with local working groups and private landowners. Moreover, these models enable the linkage of management action to collision risk, which promotes effective resource use and minimizes the inefficient strategies of mitigating collision risk randomly or everywhere (Black and Groombridge 2010). Lastly, our example threshold of >1 collision/season was somewhat arbitrary, and maps with any desired risk threshold could be constructed in a GIS to delineate areas for fence marking or moving.

Our models provide a useful tool but they should also serve as testable hypotheses, and model validation is a valuable next step because spatial extrapolation and simplifying assumptions can lead to erroneous predictions (Miller et al. 2004). A model predicting blue crane (*Anthropoides paradiseus*) power-line collision in South Africa did not successfully predict high-risk sites (Shaw et al. 2010), but the model was based on expert opinion instead of a designed field study. Our model projected predictions at the 1 \times 1-km scale onto 30-m pixels across sage-grouse breeding habitats, and with the exception of distance to lek, we assumed collision risk was independent of each pixel's position on the landscape, both of which could induce error in spatial extrapolation (Miller et al. 2004). Our models also extrapolated collision risk observed in Idaho to other western states, implicitly assuming the relationship observed between collision risk, terrain ruggedness, and lek location remains similar in other regions (Miller et al. 2004). However, prioritizing management actions using the best available science is better than proceeding with mitigation in an unorganized fashion (Miller et al. 2004). Moreover, our results are predicated on the presence of fences at each 30-m pixel. Thus, the true total area (i.e., no. of ha) of high collision risk in sage-grouse breeding areas will likely be considerably less than our models predicted because fences are not present at all sites. Lastly, our spatially-explicit models do account for removal error, but do not account for detection error and thus produce predictions of relative

collision frequency over a breeding season. Predictions of relative collision frequency and cross-scale extrapolation of predictions complicate the assessment of demographic effects on grouse populations. Hierarchical statistical models for avian-collision data incorporating both detection and evidence-removal error are a necessary next step that should facilitate predictions of the absolute number of collisions over time as a function of covariates.

We caution readers against making direct inferences to population-level benefits resulting from reduced sage-grouse collision risk. We cannot say, for example, how many sage-grouse would be added to a population by reducing collisions because we lack demographic data to know whether populations can compensate for mortality via increased productivity. Population-level impacts of sage-grouse fence collision also likely depend on proportional mortality of male and female grouse, which is currently unknown (Stevens et al. 2012a). Moreover, the ability to compensate for collision mortality probably varies spatially, further complicating our ability to predict the number of birds added to a population as a result of fence-marking efforts. Future work addressing demographic consequences of sage-grouse collision and the conditions under which we would expect additive collision mortality should be a research priority.

MANAGEMENT IMPLICATIONS

These findings help guide implementation of the Natural Resources Conservation Service's Sage Grouse Initiative and provide decision support to others working in sage-grouse conservation. We attempted to bridge the research-implementation gap by applying our model to 4,684 known lek sites across 10 western states, and provided our GIS-based tool to Natural Resources Conservation Service practitioners and the state wildlife managers responsible for management of sage-grouse populations. Managers can use this tool to identify high-risk fences and to build new fences away from high-risk areas while still accomplishing grazing objectives. To facilitate use we also developed a how-to instructional guide and conducted multiple web-based training sessions. Lastly, we made our decision-support tool

available to the Bureau of Land Management, the federal agency managing >50% of remaining sage-grouse habitats and currently revising their land-use plans for lands that include sage-grouse habitat. We encourage those interested in sage-grouse conservation to contact their state fish and wildlife agency to learn how to obtain a copy of the decision-support tool. Lastly, we remind managers that fence marking in other seasonal habitats, including areas of high sage-grouse concentration during winter, could potentially reduce fence strikes, but resulting benefits have not been measured.

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STUDY I, Job V: Greater Sage-grouse Lek Attendance Rates in Southern Idaho: Visibility Bias for Sage-Grouse Lek Counts

This study has been completed and the results are available in a doctoral dissertation. The following is being prepared for publication.

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ABSTRACT: Declining breeding populations of greater sage-grouse necessitates careful assessment and accurate estimates of trends at the population level. The primary method for monitoring sage-grouse is by comparing numbers of males observed at groups of traditional lek sites among years. The sightability of male sage-grouse on leks is likely < 1 for numerous reasons. The result is termed visibility bias and has generally been overlooked when considering lek counts. Our objectives were to identify variables that influence sightability of male sage-grouse during lek route surveys and to develop a model to correct for visibility bias. We conducted simultaneous counts of sage-grouse from a truck using standard lek-counting procedures and from blinds positioned ≤ 20 m from the edge of the leks. We built candidate models for predicting visibility bias using logistic regression with variables specific to the lek and time of observation. Sightability in our study varied from $< 50\%$ under certain circumstances to 100% under others. Our analysis revealed strong evidence that sightability of sage-grouse was negatively correlated with lek area, visual obstruction, distance, and time. Alternatively, hen presence increased sightability, as did direct sunlight until 30 min past sunrise. Estimating and correcting for variation in these counts due to visibility bias should improve their power to detect differences among populations or through time, making results more reliable and defensible.

INTRODUCTION

Breeding populations of greater sage-grouse (*Centrocercus urophasianus*, hereafter, sage-grouse) have declined an estimated average of 33% across their range over recent decades and average declines have been estimated at 2.0% per year from 1965-2003 (Connelly and Braun 1997, Connelly et al. 2004). As a result of this and other factors, sage-grouse were considered warranted for protection under the Endangered Species Act of 1973 by the U.S. Fish and Wildlife Service in 2010; however, this consideration was precluded by the need to protect species with more critical threats of extinction (USDI-FWS 2010). This classification requires that the status of the species be carefully assessed annually, necessitating accurate information on trends at the population level.

The primary method for monitoring sage-grouse abundance is via an index of breeding populations. Each spring, males assemble and display in groups called leks, and females visit the leks and select a male for breeding (Höglund and Alatalo 1995). Due to the conspicuousness of

displaying males and the lack of vegetative cover typical of leks, these congregations are relatively easy to locate (Schroeder et al. 1999). Furthermore, lek sites are usually traditional and persist for long periods of time (Patterson 1952, Dalke et al. 1963). The recommended protocols for indexing populations of sage-grouse involve counting males congregated at multiple lek sites along established travel routes (i.e., lek routes; Patterson 1952, Connelly et al. 2000, Connelly et al. 2003). Connelly et al. (2003) recommended each route be run a minimum of 4 times each spring; the maximum number of males per lek route counted on a single visit is used to estimate inter-annual trends within lek routes and regional populations. The value of these indices rely on the following assumptions: (1) the number of males counted on leks is proportional to total male population; (2) the proportion remains constant among years; and (3) the detection probability is the same among years and for all observers (Anderson 2001, White 2005).

We define sightability of sage-grouse as the probability that an individual is included in a lek count, given that it is present at the lek. The sightability of male sage-grouse on leks is likely < 1 for numerous reasons. The result is termed visibility bias and has generally been overlooked when considering lek counts. Ignoring visibility bias likely results in counts that are biased low and if visibility bias varies among leks or in time, the underlying assumption that the proportion counted remains constant may become invalid (Pollock et al. 2002). Additional noise introduced into the index by visibility bias can make accurate assessment of population trends more difficult (Williams et al. 2002). Johnson (2008) argued that detectability of animals does not necessarily need to remain constant for an index to be useful, as long as variation in detection is less than the change in population sought to be detected by the index. While it may not be necessary to correct for sightability, understanding the magnitude of effect sightability has on lek counts, at a minimum, is necessary for the index to be of value. Furthermore, understanding sources of visibility bias should be useful for improving guidelines for conducting lek routes.

Visibility bias has been considered for a number of ungulate (Steinhorst and Samuel 1989, McIntosh et al. 2009, Rice et al. 2009) and waterfowl species (Prenzlów and Lovvorn 1996, Pearse et al. 2007, Vrtiska and Powell 2011) that are routinely surveyed via aerial counts; however, little consideration has been given to accounting for bias in sightability of sage-grouse during lek counts. Our objectives were to identify variables that influence sightability of male sage-grouse during lek route surveys and to develop a model to correct for visibility bias that incorporates detection rates in order to provide unbiased estimates of the number of male sage-grouse attending leks.

METHODS

Study site

We collected lek data on Brown's Bench and surrounding areas located in Twin Falls County in south central Idaho. Brown's Bench is bordered to the east by Salmon Falls Creek Reservoir and to the west by an area of rolling hills locally known as Monument Springs. This area receives approximately 24 cm of precipitation annually and ranges in elevation from 1524 m to 2300 m. Major cover types include little sagebrush (*Artemisia arbuscula*) /black sagebrush (*A. nova*) /grass, Wyoming big sagebrush (*A. tridentata wyomingensis*)/grass, mountain big sagebrush (*A. t. vaseyana*)/grass, mountain shrub, and crested wheatgrass (*Agropyron cristatum*) seedings. Sub-dominant cover types include aspen (*Populus tremuloides*) woodland, mountain mahogany

(*Cercocarpus montanus*) woodland, and wet meadow/riparian (Hironaka et al. 1983, Klott et al. 1993). The majority of the study area is owned by the Bureau of Land Management and is primarily managed for cattle grazing.

Ground Surveys

We located leks and conducted counts from a truck on all known leks in our study area on a weekly basis. We used standard lek-counting procedures (Connelly et al. 2003) beginning approximately the third week of March and continuing through the end of May in 2007-2009. In each year of the study, we positioned blinds on a different subset of these leks. Blinds were positioned ≤ 20 m from the edge of the leks in the thickest brush available and were positioned a minimum of 1 week prior to use. Observers entered the blind ≥ 1.5 hour before sunrise to reduce the chance of flushing birds. We used a hand-held, 2-way radio to coordinate timing of counts between the blind observer and the observer in the truck. Both observers used 20-60X spotting scopes and the truck observer performed 3 complete counts per Connelly et al. (2003), while the blind observer independently counted and watched for birds entering or leaving the lek or his or her field of view. Immediately before or after the count, the truck observer recorded the temperature with a hand-held thermometer, and average and maximum wind speeds over a 15 second interval with a Kestrel 1000 Pocket Wind Meter (Boothwyn, PA). Time of count, estimated cloud cover, whether or not there was direct sun on the lek during the count, and if any hens were observed by the truck observer were also recorded. If conditions included wind > 15 kph or precipitation, lek routes were abandoned and re-run when conditions allowed (Connelly et al. 2003).

In mid-May, when all lekking activity ceased, we revisited each lek in our sample to measure visual obstruction, area of lek, and distance from truck observation point to center of lek. We used a Robel pole with 1-decimeter increments clearly marked (Robel et al. 1970) at each of 25 systematically selected random points, while an observer sat in a truck parked at the truck observation point and read the pole using the same spotting scope used during lek route counts. This measurement accounted for visibility obstructed by vegetation as well as topography of the leks relative to the observation point. Edge of the lek was subjectively determined based on outermost locations of displaying sage-grouse by ≥ 2 observers from earlier in the season. We used the “edges” of the lek to calculate the geographic center, as well as lateral and perpendicular radii for calculating the area of an ellipse used to estimate the area of the lek. We used the compass bearing of the center of the lek and corrected for the slight change in the earth’s tilt over the season to calculate the bearing of observation relative to the rising sun for each day of observation at each lek.

Data analysis

We hypothesized that visibility bias would be affected by 3 categories of variables: 1) lighting conditions, 2) conditions of the lek and observer, and 3) variables that might influence the behavior of male sage-grouse on the lek and thereby affect the probability of individual birds being observed. For lighting conditions, we hypothesized that increased cloudiness, specifically after sunrise, would block light and reduce visibility, direct sunlight on leks would improve sightability, and observations made closer to the bearing of the rising sun would result in decreased sightability. Cloudiness was estimated at time of truck observation as % of sky obscured by clouds. We assumed the 3 variables for light conditions might interact with time,

which we standardized by sunrise. We did not include cloudiness or direct sunlight in our models without the time interaction because it was not possible to observe direct sunlight before sunrise, and we did not assume that the effect of cloudiness would be the same before and after sunrise.

Lek and observer-specific variables included the average visual obstruction score, area of the lek, distance to the center of the lek, and observer experience. We hypothesized that increased distance from the observation point to the lek, increased visual obstruction, and leks with a greater area would result in lower visibility. Numerous authors report decreases in visibility bias of individuals or groups of animals with increasing observer experience (Leresche and Rausch 1974, Ayers and Anderson 1999, Garel et al. 2005). We included the experience of the truck observer calculated as the number of lek counts he or she performed in the study area prior to the specific observation and hypothesized that visibility bias would decrease as the number of lek counts increased.

Activity of the individual or group under consideration has been reported to affect visibility bias for large mammals such as elk (McIntosh et al. 2009). Male sage-grouse display rate has been reported to be highly correlated with hen presence (Scott 1942, Hartzler 1972); we hypothesized that hens present on the lek would increase displaying activity of males, and therefore increase detection. Sherfy and Pekins (1995) suggested that sage-grouse may seek refuge in or near vegetation during high winds and cold temperatures. This behavior could result in individuals leaving the lek during severe weather conditions, but could also result in individuals moving to areas of the lek with greater cover and becoming less active. We hypothesized that sage-grouse sightability would decrease with increasing wind, decreasing temperature, or as temperatures became increasingly cooler than expected (temperature deviance). Temperature deviance was designed to account for any variation due to extreme temperatures corrected for time of season, which we calculated as the difference between the observed temperature and the expected temperature for the specific day and time of each lek observation. We used hourly temperatures between 5:00 am to 10:00 am from 13 March through 18 May for the years 2000-2010 to build a regression model with the following variables: time, time², date, date², and time×date to predict temperature. These data were collected 60 km from our field site at the Joslin Field – Magic Valley Regional Airport, which was the nearest weather station that recorded temperature on an hourly basis (NOAA's National Climatic Data Service). We adjusted our expected temperature from the resulting prediction equation by -2°C since our observed temperatures recorded during our resighting occasions were on average 2°C lower than those measured at the weather station.

We built candidate models for predicting visibility bias in 2 stages. In the first step, we created models for each of the 3 previously mentioned categories based on published literature and our knowledge of sage-grouse and lek observations. We avoided multicollinearity among the covariates by calculating tolerance values from the reciprocal of the variation inflation factor using the REG procedure in SAS and excluded variables with tolerances < 0.4 (Neter et al. 1990). We used Akaike's Information Criterion (AIC) to compare models and included variables from models for the 3 categories in the first stage with $\Delta AIC < 2$ of the best fit model to create a new set of candidate models for the final stage (Burnham and Anderson 2002).

To address the objective of identifying covariates that influence the sightability of sage-grouse on leks, we used logistic regression with the proportion of total grouse that were actually observed from the truck observation as the response variable. We performed analyses using the GENMOD procedure in SAS, using a binomial probability distribution and logit link function. We assessed the predictive abilities of the top supported model with leave-one-out cross-validation. This is an iterative method that uses each single observation to validate the model, where the remaining observations are used to fit the model.

We developed a second model that balanced predictive performance with simplicity for the purpose of correcting raw counts. Therefore, we used the top supported model and eliminated from it variables deemed labor-intensive or subjectively defined. This reduction resulted in the elimination of area, visual obstruction, experience, and temperature deviance. We then selected the model with the lowest AIC value that did not include one of the eliminated variables, and used leave-one-out cross-validation to judge its relative predictive ability.

RESULTS

We conducted a total of 73 lek counts for this analysis; 15 on 3 leks in 2007, 24 on 4 leks in 2008, and 34 on 5 leks in 2009. In total, 911 individual male sage-grouse were observed from the truck, out of 1160 that were counted from the blind, for an average sightability of 0.79. Individual lek counts resulted in an average bias of -3.4 sage-grouse per lek, and ranged between perfect counts 36% of the time and an extreme case where only 3 of 20 sage-grouse were observed.

The first stage of model selection revealed the lighting model with time, direct sun, cloud, and time interacting with both direct sun and cloud as having the best fit (Table 1). Considering the lek and observation-specific variables, the model with lek area, visual obstruction, and distance resulted in the lowest AIC value, though the model with lek area, distance, and observer experience was equally parsimonious. The best supported model with behavior variables included presence of one or more hen detected from the truck. The best overall model for explaining visibility included the covariates of lek area, visual obstruction, distance, hen presence, time, direct sun, and an interaction term for time and direct sun (Table 1).

Table 1. Best supported models for the first stage of model fitting for 3 categories of variables, the best overall model from the second stage of modeling, and the reduced correction model for explaining visibility bias of greater sage-grouse on Browns Bench, Idaho, 2007-2009.

Category	Model Description	K	AIC
Null	Intercept only	1	430.7
Lighting	Time+DirSun+Cloud+(Time*DirSun)+(Time*Cloud)	6	367.0
Lek/Observer	Area+VO+Distance	4	310.5
	Area+Distance+Experience	4	312.2
Behavior	Hen	3	415.5
Overall	Area+VO+Distance+Time+DirSun+(Time*DirSun)+Hen	11	280.8
Correction	Distance+Time+DirSun+(Time*DirSun)+Hen	9	294.5

^a Abbreviations: Time = time relative to sunrise, DirSun = direct sunlight on the lek, VO = visual obstruction, and Hen = hen(s) present,.

There was strong evidence that sightability of sage-grouse was negatively correlated with area, visual obstruction, distance, and time. Alternatively, hen presence increased sightability (Table 2).

Table 2. Regression coefficients, unconditional standard errors (SE), and 95% confidence intervals (CI) from the top supported sightability regression model for sage-grouse on leks on Browns Bench, Idaho, 2007-2009.

Variable	Coefficient	SE	95% CI	
			Lower	Upper
Intercept	5.75	0.716	4.35	7.16
Area	-0.0260	0.00893	-0.0435	-0.0085
VO	-0.217	0.100	-0.413	-0.0205
Distance	-0.00115	0.000363	-0.0019	-0.0004
Time	-0.0265	0.00611	-0.0385	-0.0146
Direct Sun	-0.667	0.289	-1.23	-0.101
Time x Direct Sun	0.0230	0.00741	0.0084	0.0375
Hen Presence	-0.855	0.244	-1.33	-0.377

We predicted the sightability (proportion counted) of sage-grouse for the observed range of each variable separately while holding other variables at their observed mean, with no hens present or direct sun, unless otherwise noted (Fig. 1). The observed means were for a lek 384.4m away with an area of 16,776 m², a visual obstruction of 7.12, observed at 9.6 minutes past sunrise. These mean values resulted in predicted sightability of 85.5%. Sightability was predicted to decrease 20% (from 89% to 69%) as distance to the lek increased from 130 m to 1,225 m (Fig. 1A). Visual obstruction had an inverse relationship with sightability; sightability decreased from 94% to 76% as visual obstruction increased from 30 cm to 100 cm (Fig. 1B). Predicted sightability at the smaller range of lek area was 89%, but decreased to 77% for our largest leks (Fig. 1C). When hens were detected on the lek, sightability was predicted to increase 7% at 30 min before sunrise and 10% at 90 min past sunrise (Fig 1C). As observations were made later in the morning, sightability was predicted to decrease slightly from 87% at 30 minutes before sunrise to 82% at 90 minutes past sunrise. However, with direct sun shining on the lek, sightability was predicted to decrease from 92% at sunrise to 54% at 90 min past sunrise (Fig. 1D).

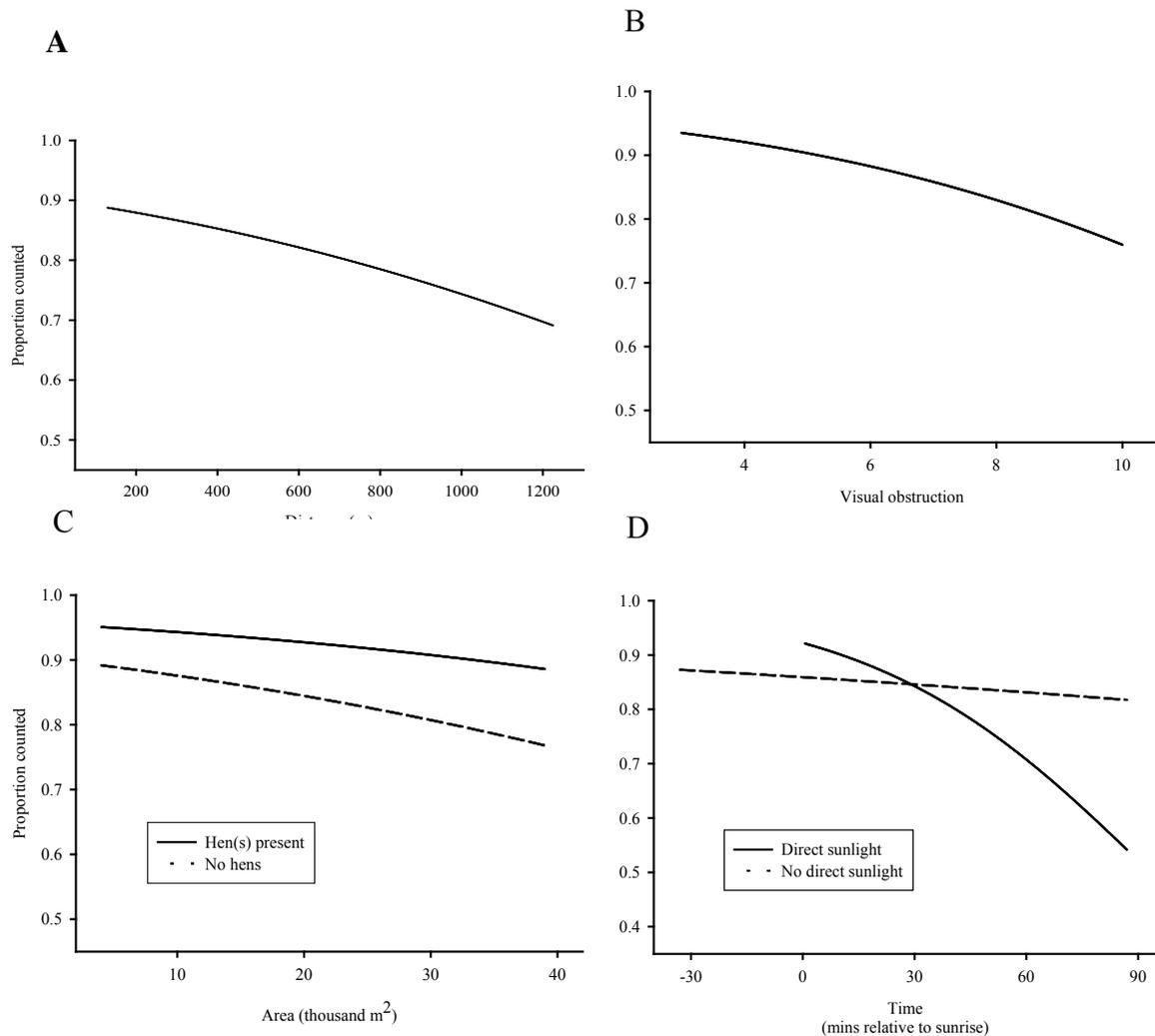


Figure 1. Sightability of sage-grouse from a truck count predicted from the top supported logistic regression model including A) distance to lek center B) visual obstruction, C) lek area and hen presence, and D) time and direct sunlight, and as predictor variables, Browns Bench Idaho, 2007-2009. Sightability was calculated for the observed range of each variable as proportion counted and by holding all other variables at their observed mean, with no hens present or direct sun, unless otherwise noted. The observed means were 10 sage-grouse counted from the truck, on a lek 130.5 m away with an estimated area of 16,776 m², a visual obstruction of 7.12, with 36% cloud cover at 9.6 minutes past sunrise.

Model validation and correction model development

The leave-one-out cross-validation revealed an average bias in predicted count of 0.071 sage-grouse per lek and a root-mean-square-error (RMSE) of 2.97. The model with the lowest AIC value that did not include variables we determined were labor intensive or subjectively defined included time, direct sun, time x direct sun, distance, and hen presence (Table 1 above).

Relationships of covariates remaining in the reduced model were similar to those from the overall model (Table 3). Cross-validation of the reduced correction model revealed an average bias in the predicted count of 0.016 and a RMSE of 2.73.

Table 3. Reduced logistic regression coefficients, standard errors (SE), and 95% confidence intervals (CI) from sage-grouse sightability on leks on Browns Bench, Idaho, 2007-2009.

Variable	Coefficient	SE	95% CI	
			Lower	Upper
Intercept	3.98	0.372	3.25	4.71
Distance	-1.00213	0.00022	-0.0026	-0.0017
Time	-0.0303	0.00595	-0.420	-0.0017
Direct Sun	-0.716	0.284	-1.27	-0.159
Time x Direct Sun	-0.0224	0.00729	0.0081	0.0367
Hen Presence	-0.850	0.241	-1.32	-0.378

DISCUSSION

We estimated sightability of sage-grouse and built models to identify variables that help explain visibility bias for leks on Brown's Bench, Idaho. Our data indicated that sightability improved for leks of smaller area, for leks with lower visual obstruction, leks located closer to the truck observer, and for counts conducted earlier in the morning. Visibility also appeared to improve when hens were present. Direct sunlight on the lek had a positive effect on sightability for the first 30 min past sunrise, after which time there was a negative relationship.

The inclusion of distance in our models is not surprising; the premise that individual animals further away are more difficult to see is well understood in the field of wildlife management. The discipline of distance sampling is based on estimating the assumed relationship between the probability of detection and the distance of the animal for estimating densities (Williams et al. 2002). Likewise, the inclusion of visual obstruction was also expected and easily explained. Where sage-grouse have greater likelihood for concealment, either by brush or topography, more individuals will be missed.

The relationship of lek area in our model suggests larger leks in our study area had poorer sightability. While our larger leks were generally associated with a greater number of attending males, these leks also had lower density of displaying males. It is likely that solitary males, possibly on the lek periphery and not associated with a densely congregated group are often missed during lek counts.

According to our model predictions, sage-grouse are most visible 0.5 hours before sunrise when lighting conditions seem relatively poor. The negative relationship between time and visibility suggests sage-grouse become less active as the morning progresses, which supports the results of

numerous authors (Scott 1942, Rogers 1964, Hjorth 1970, Schroeder et al. 1999). The positive correlation between hen presence and sightability also support the hypothesis that male sage-grouse are more active when females are present.

The apparent positive effect of direct sunlight on sightability for the first 30 min after sunrise is likely a result of improved lighting. However, this hypothesis was not supported by our data for the remaining hour that we observed leks. This phenomenon may be explained from a behavioral response. Due to their display behavior and plumage, lekking sage-grouse are highly conspicuous, making them extremely vulnerable to predation by visual predators. Golden eagles (*Aquila chrysaetos*) are considered the primary diurnal predator of sage-grouse, and accounts of both successful and unsuccessful attempts of golden eagles attacking male sage-grouse that were attending leks are numerous (Scott 1942, Hartzler 1974, Patterson 1952, Bradbury et al. 1989). In response to golden eagles seen overhead, sage-grouse have been observed to crouch low and remain still (Patterson 1952, Hartzler 1974), suggesting inactivity is a predator avoidance behavior. Bergerud and Gratson (1988) noted that male mating display is costly and should only be done when the potential benefits outweigh the costs. Golden eagles decrease their activity of hunting during periods of overcast (Dekker 1985), which could be perceived as potentially less risky conditions for display by sage-grouse. It is, then, reasonable to assume sage-grouse are less active under brighter conditions when the perceived risk of predation is greater, which could explain why we detected a smaller proportion of males on leks later in the mornings with direct sun.

Our data do not support the hypothesis that observer experience affects observation bias of lekking sage-grouse. Numerous authors examining sightability of animals from aerial surveys have found observer experience to be an important consideration (Leresche and Rausch 1974, Ayers and Anderson 1999). This discrepancy might be due to the differences between ground and aerial survey methods. Technicians conducting lek routes are only limited by the time restraint recommended by Connelly et al. (2003) of counting all leks in a route within a 1.5 hour window. Technicians for our project generally were only expected to count 3 leks on a given morning. This allowed for sufficient time to travel between leks and ample time to count sage-grouse such that inexperience may be compensated for by increasing time spent at a given lek. Aerial observations, in contrast, are limited by time based on the speed and height of the aircraft, variables that are typically held constant for the purpose of standardizing protocol.

We did not see evidence from our analysis that sage-grouse decrease their level of activity, thereby decreasing their detection in windy or cold conditions. This result was not surprising, considering we limited our observations to mornings with winds < 15 kph (Connelly et al. 2003) and the mean temperature during our observations was 4°C with a minimum of -6.7° C. Under more extreme conditions, male sage-grouse might be expected to decrease display activity if they occupy the lek at all (Sherfy and Pekins 1995).

The reduced correction model only contains variables that are easily defined and collected (distance, time, direct sun, and hen presence). Considering the cross-validation diagnostics, the predictive performance of this model for our study area was generally good and even slightly better than the model with greatest AIC support. We propose this reduced model be tested and further developed such that the potential area of application is broadened to include entire sage-

grouse management zones proposed by Stiver et al. (2006) and possibly the entire distribution of the species. We caution against the use of our lek count correction model outside our study population without further testing. Our model was built with data that do not necessarily represent conditions outside the current extent. For example, our average lek size in number of attending males was 16 (range 2 – 50), which is smaller than for many populations, and our study site likely receives less snowfall than much of the species' distribution. Broadening the range of variables used to develop a correction model will likely change the magnitude or even type of relationship assumed between explanatory and response variables in the model.

Management implications

Sage-grouse populations are typically monitored through most of their range with annual lek-route counts, particularly for long-term trend estimation. Due to the potential noise from outside sources, such as variable lek attendance probabilities and visibility bias, the utility of lek-routes for indexing populations has been questioned (Beck and Braun 1980, Emmons and Braun 1984, Zablan et al. 2003, Walsh et al. 2004). Estimating and correcting for variation in these counts due to visibility bias should improve their power to detect differences among populations or through time, making results more reliable and defensible.

The lack of consideration for visibility bias previous to this work implies sightability of lekking sage-grouse was considered high and, perhaps, constant. Our results suggest otherwise, with sightability reaching below 50% under certain circumstances and 100% under others. While the effects of visibility bias may be reduced by following the suggestions of Connelly et al. (2003) of repeating each route a minimum of 4 times each spring, accounting for this source of bias will improve reliability of lek route counts. We recommend biologists test our sightability model to further develop and improve its generality across the distribution of sage-grouse. Application of the correction model we propose requires the additional data of time of count, direct sun, distance between observer and center of lek, and presence of hens. Many of these covariates are likely included on data sheets used by agencies involved with monitoring breeding populations of sage-grouse. We recommend these and any other variables identified as useful for correcting visibility bias become standard data collected during lek routes as they will require little additional time, but have the potential to improve the power of the index.

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Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales

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Abstract. Animal habitat selection is a process that functions at multiple, hierarchically structured spatial scales. Thus multi-scale analyses should be the basis for inferences about factors driving the habitat selection process. Vertebrate herbivores forage selectively on the basis of phytochemistry, but few studies have investigated the influence of selective foraging (i.e., fine-scale habitat selection) on habitat selection at larger scales. We tested the hypothesis that phytochemistry is integral to the habitat selection process for vertebrate herbivores. We predicted that habitats selected at three spatial scales would be characterized by higher nutrient concentrations and lower concentrations of plant secondary metabolites (PSMs) than unused habitats. We used the Greater Sage-Grouse (*Centrocercus urophasianus*), an avian herbivore with a seasonally specialized diet of sagebrush, to test our hypothesis. Sage-Grouse selected a habitat type (black sagebrush, *Artemisia nova*) with lower PSM concentrations than the alternative (Wyoming big sagebrush, *A. tridentata wyomingensis*). Within black sagebrush habitat, Sage-Grouse selected patches and individual plants within those patches that were higher in nutrient concentrations and lower in PSM concentrations than those not used. Our results provide the first evidence for multi-scale habitat selection by an avian herbivore on the basis of phytochemistry, and they suggest that phytochemistry may be a fundamental driver of habitat selection for vertebrate herbivores.

Key words: *Artemisia spp.*; *Centrocercus urophasianus*; Greater Sage-Grouse; habitat selection; herbivory; monoterpenes; plant secondary metabolites; sagebrush; selective foraging; south-central Idaho, USA; spatial scale.

INTRODUCTION

Ecological processes function simultaneously within multiple, hierarchically structured spatial scales (Wiens 1989). Consequently, inferences about the factors that influence ecological pattern and process are conditional on the spatial scale under investigation (Kristan 2006). Coarse-scale studies may indicate the importance of a particular set of factors, whereas fine-scale studies may indicate influences of an entirely different set of factors (Wiens et al. 1986). Thus, a holistic understanding of ecological pattern and process requires information from multiple scales. Animal habitat selection is an ecological process that occurs at multiple scales (Johnson 1980). Coarse-scale measurements may yield inferences about habitat selection that differ from those made at a fine scale within the same system (e.g., Becker and Beissinger 2003). Although patterns of habitat selection at a single scale are often important, patterns observed consistently at multiple scales may provide

more robust inferences about factors fundamentally driving the habitat selection process.

Vertebrate herbivores have been the focus of an increasing number of multi-scale habitat selection studies (e.g., Moore et al. 2010, Rearden et al. 2011), which have yielded insights that may have been lacking from single-scale investigations. However, few studies have directly considered the role of diet in habitat selection by herbivores, despite the importance of food quality to individual fitness (DeGabriel et al. 2009). Herbivore diets are constrained by the nutritional and defensive chemistry of plants (Marsh et al. 2005). Although typically abundant, plants are relatively low in nutrients (White 1978) and often contain defensive compounds (i.e., plant secondary metabolites, PSMs; Dearing et al. 2005). This combination places conflicting pressures on herbivores to maximize consumption of one plant constituent (i.e., nutrients), while simultaneously minimizing consumption of another (i.e., PSMs; Dearing et al. 2005). These conflicting pressures may shape the evolution of selective foraging strategies (Bryant and Kuropat 1980), which may then influence the process of herbivore habitat selection (Moore et al. 2010).

We tested the hypothesis that the defensive and nutritional chemistry of plants fundamentally influences

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habitat selection by vertebrate herbivores. We predicted that habitats selected at multiple scales would be characterized by high-nutrient and low-PSM plants. We used an avian herbivore with a seasonally specialized diet of high-PSM plants to test our hypothesis. The Greater Sage-Grouse (*Centrocercus urophasianus*; hereafter Sage-Grouse) is an herbivorous tetraonine with a specialized diet of sagebrush (*Artemisia* spp.) leaves from late autumn through early spring (Wallestad et al. 1975). Sagebrush foliage contains relatively high concentrations of PSMs (e.g., monoterpenes, sesquiterpene lactones, phenolics; Kelsey et al. 1982) and previous work has suggested that Sage-Grouse browse sagebrush selectively with respect to nutrients and PSMs (Remington and Braun 1985, Welch et al. 1988). We evaluated the effects of nutrients and PSMs on Sage-Grouse habitat selection at three spatial scales: (1) selection of sagebrush habitat type, (2) selection of patches within a given habitat type, and (3) selection of plants within a given patch (i.e., diet selection). Here, we provide the first evidence for multi-scale habitat selection by an avian herbivore on the basis of phytochemistry.

METHODS

Study site

We conducted this study during the winter of 2010–2011 with a Sage-Grouse–sagebrush system in a ~19 000 ha area of south-central Idaho, USA (42°11' N, 114°46' W). The study area was between 10 and 316 times the size of reported Sage-Grouse winter home ranges, depending on the individual bird and home range estimation method (Schroeder et al. 1999). Dominant vegetation at the study site was a mosaic of black sagebrush (*Artemisia nova*) and Wyoming big sagebrush (*A. tridentata wyomingensis*) stands with a mixture of native grasses, exotic grasses, and herbaceous vegetation in the understory. Elevations ranged from approximately 1550 m to 1750 m and average annual precipitation was 26.3 cm. Winter high temperatures averaged 4.6°C and winter low temperatures averaged –5.9°C. Maximum snow depth observed during the study was 7.5 cm, but snow cover was absent during the majority of the study period.

Field methods

We marked 38 Sage-Grouse with ~17 g necklace-style VHF radio transmitters during 2010 and 2011. During each of three winter sampling periods (23–30 January 2011, 6–13 February 2011, and 8–14 March 2011), we used radiotelemetry to locate randomly selected Sage-Grouse flocks containing radio-marked birds during daylight. Radio-marked grouse were located one to three times each during the study, but individuals were never located repeatedly within the same sampling period. We collected samples of browsed and non-browsed sagebrush plants at each flock location (hereafter, “used patch”). Browsed plants were defined as those with evidence of at least 10 fresh Sage-Grouse

bite marks (Appendix A: Fig. A1). Non-browsed plants were defined as plants with (1) no evidence of Sage-Grouse browsing and (2) evidence of Sage-Grouse presence (tracks, droppings, and browsed plants) within 0.5 m of the plant. At each used patch ($n = 55$), clippings from three browsed and three non-browsed plants were collected and pooled to form a single browsed sample and a single non-browsed sample. Additionally, we generated random coordinates ($n = 55$) constrained by the boundaries of the study area. At each set of random coordinates (hereafter, “random patches”), we collected clippings from the three closest sagebrush plants and pooled them into a single sample. We did not observe evidence of Sage-Grouse presence (e.g., browsed sagebrush, fecal droppings, tracks) at random patches. All samples collected within a given used or random patch were conspecific with one another and with the dominant sagebrush taxon (Wyoming big sagebrush or black sagebrush). Structural vegetation measurements can affect Sage-Grouse habitat use (Connelly et al. 2000, Hagen et al. 2007), so we measured shrub canopy cover (Wambolt et al. 2006) and height along two perpendicular 30-m transects at each used and random patch to account for this effect.

Laboratory methods

We stored sagebrush samples in a –20°C freezer, ground the leaves in liquid nitrogen using a mortar and pestle, and divided each sample into three subsamples. The first subsample was used to quantify individual monoterpenes via headspace gas chromatography (Appendix B). We identified individual monoterpenes using co-chromatography with known standards. The second subsample was used in a colorimetric assay to quantify total phenolic concentrations (Appendix C). The third subsample was dried and shipped to Dairy One Forage Laboratory (Ithaca, New York, USA) for analysis of crude protein content.

We used crude protein as a nutrient variable because it can affect herbivore foraging behavior and reproductive success (Mattson 1980). Nutrient concentrations were quantified as percentage of dry mass. We chose monoterpenes and phenolics as PSM variables because both classes of compounds exert deleterious effects (e.g., toxicity, increased energy expenditure, nutrient binding) on herbivores (Dearing et al. 2005) and occur in relatively high concentrations in sagebrush (Kelsey et al. 1982). Monoterpenes were quantified as area under the gas chromatogram curve/100 µg dry mass (AUC/100 µg; Appendix B), and total phenolic concentrations were quantified as µmol gallic acid equivalents/g dry mass (Appendix C).

Statistical analysis

Prior to fitting models, we used Fisher's exact test to examine our a priori belief that Sage-Grouse used black sagebrush habitat more than expected on the basis of availability. Starting with one nutrient covariate (crude

TABLE 1. Model-averaged odds ratio estimates and 95% confidence limits for covariates in final Sage-Grouse habitat-selection models at each of three spatial scales, south-central Idaho, USA.

Scale and covariate	Odds ratio	95% CL	
		Lower	Upper
Habitat type			
Crude protein (%)	0.37	0.10	0.83
Total monoterpenes (AUC/100 μg)	0.89	0.79	0.98
Shrub height (cm)	0.82	0.67	0.91
Patch			
Crude protein (%)	1.69	1.12	2.55
Unknown #1 (AUC/100 μg)	0.60	0.37	0.96
1,8-cineole (AUC/100 μg)	1.04	0.90	1.20
Plant			
Crude protein (%)	2.02	1.03	3.98
1,8-cineole (AUC/100 μg)	0.82	0.70	0.97
α -pinene (AUC/100 μg)	0.88	0.75	1.03
Unknown #1 (AUC/100 μg)	0.84	0.66	1.08

Notes: At the habitat scale, the response is the log odds that a habitat is black sagebrush. At the patch scale, the response is the log odds that a patch within black sagebrush habitat is used. At the plant scale, the response is the log odds that a plant within a used patch is browsed.

protein), seven PSM covariates (Appendix B: Table B1), and two structural covariates (shrub height, shrub canopy cover), we removed collinear variables ($|r| > 0.7$, variance inflation factors > 3.0) and fit binary logit models separately at each of three spatial scales. To identify factors associated with selection of habitat type, we treated habitat type (Wyoming big sagebrush or black sagebrush) at random patches as a binary response and the nutrient, PSM, and structural covariates as continuous predictors. To assess selection of patches within a given habitat type, we treated patch type (used or random) as a binary response and the nutrient, PSM, and structural covariates as continuous predictors. Covariate values for used patches were the means of all sagebrush samples (browsed and non-browsed) collected therein. To assess selection of plants within a given patch (i.e., diet selection), we treated plant type (browsed or non-browsed) as a binary response and the nutrient and PSM covariates as continuous predictors. For the analysis of plant selection, we used conditional logit models to control for variation among patches (Compton et al. 2002).

We used a hierarchical information-theoretic approach to model selection and model averaging (Burnham and Anderson 2002, sensu Doherty et al. 2008) within each spatial scale. First, we identified the variables that best represented each of the three predictor categories (nutrient, PSM, structure) by comparing Akaike's Information Criterion values with sample size bias adjustment (AIC_c) for all variable combinations within each category (Appendix D: Tables D2–D4). Within the PSM category, we included total monoterpene concentration for comparison with indi-

vidual monoterpene covariates, but restricted it to a single-predictor model because of its lack of independence with individual monoterpene covariates. Covariates from the top models in each predictor category were then included in a final round of model selection unless they were ranked below the null (intercept-only) model (Appendix D: Table D1). We weighted final models within 2 AIC_c units of the top model (i.e., $\Delta AIC_c \leq 2$) by Akaike model weight (w_i) to derive model-averaged parameter estimates, and we used unconditional variance for estimating 95% confidence intervals (Burnham and Anderson 2002). To evaluate model performance, we used 10-fold cross validation to estimate classification accuracy for the top model at each spatial scale. Statistical analyses were conducted in R version 2.14 (R Development Core Team 2011) and SAS version 9.2 (SAS Institute 2008).

To screen for cross-scale correlations between spatial scales, we computed bivariate correlation coefficients between scales for all covariates that appeared in top models. We considered $|r| > 0.7$ as indicative of significant cross-scale correlation. Additionally, we plotted mean values partitioned by sample category (browsed, non-browsed, random) for covariates shared among top models at the patch and plant scales. Because the covariate values representing the phytochemistry of a given patch were composites of the values of browsed and non-browsed plants within that patch, we expected to see similar values for random and non-browsed samples (i.e., the sample categories not selected by Sage-Grouse at the patch and plant scales, respectively) if our patch-scale inferences were artifacts of plant-scale selection.

RESULTS

Selection of habitat type

Sage-Grouse used black sagebrush habitat more than expected on the basis of availability (Fisher's exact test: $P < 0.0001$, odds ratio = 27.8; Appendix E: Table E1). The single habitat-type model with $\Delta AIC_c \leq 2$ included one nutrient covariate (crude protein), one PSM covariate (total monoterpene concentration), and one structural covariate (shrub height; Appendix D: Table D2), with an estimated classification accuracy of 94%. Wyoming big sagebrush contained higher total monoterpene concentrations than black sagebrush (Table 1, Fig. 1). Additionally, Wyoming big sagebrush was characterized by higher crude protein and greater shrub height than black sagebrush (Table 1, Fig. 1). This height difference was the only difference in shrub structure observed at any spatial scale during the study.

Selection of patches

The two final patch-scale models with $\Delta AIC_c \leq 2$ contained one nutrient covariate (crude protein) and two PSM covariates (unknown #1, 1,8-cineole; Appendix D: Table D3). However, the odds ratio estimate for 1,8-cineole differed only slightly from 1 (i.e., logit

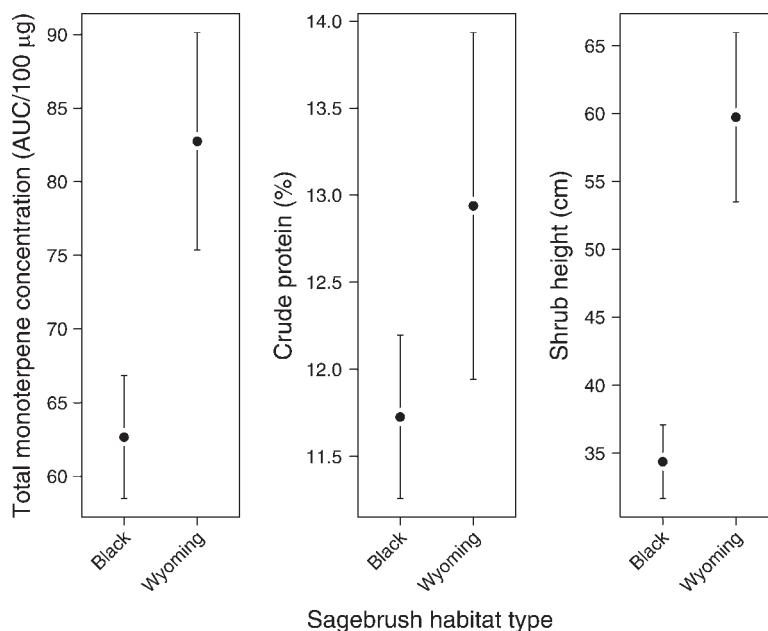


FIG. 1. Univariate comparisons (mean and 95% confidence interval) of total monoterpene concentration (AUC/100 µg dry mass), crude protein (%), and shrub height (cm) for black sagebrush (*Artemisia nova*) and Wyoming big sagebrush (*A. tridentata wyomingensis*) habitats during winter in south-central Idaho, USA. AUC refers to area under the gas chromatogram curve.

coefficient ≈ 0), with a broad confidence interval that substantially overlapped 1 (Table 1). Thus, we concluded that 1,8-cineole lacked inferential value and excluded it from further consideration at the patch scale. The estimated classification accuracy for the top model was 78%. The odds of patch use increased by 69% for each 1% increase in crude protein, whereas the odds of patch use decreased by 40% for each 1 AUC/100 µg increase in

an unidentified monoterpene (unknown #1; Table 1, Fig. 2A).

Selection of plants

The four final plant-scale models with $\Delta AIC_c \leq 2$ contained one nutrient covariate (crude protein) and three PSM covariates (unknown #1, α -pinene, 1,8-cineole; Appendix D: Table D4). Odds ratio estimates

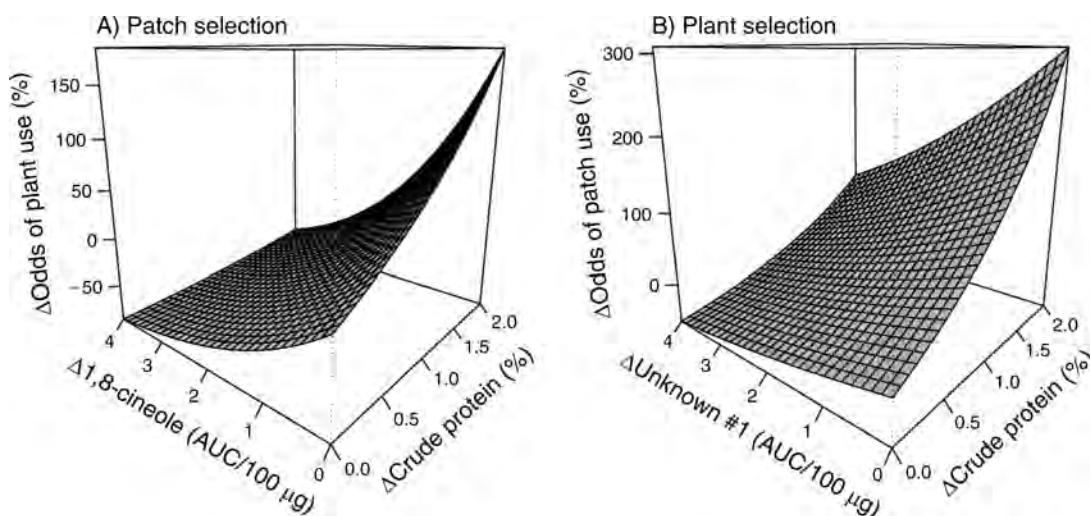


FIG. 2. Response surface depicting the additive effects of increasing plant secondary metabolites (PSM) and nutrient concentrations on the odds of Greater Sage-Grouse (*Centrocercus urophasianus*) use of (A) patches and (B) plants during winter in black sagebrush (*Artemisia nova*) habitat, south-central Idaho, USA. Changes in the odds of use were calculated using the top covariates within each spatial scale while holding all other covariates constant.

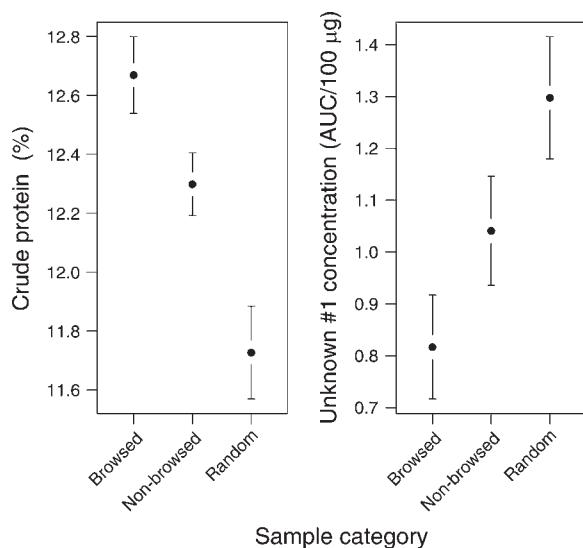


FIG. 3. Values (mean \pm SE) partitioned by sample category (browsed, non-browsed, random) for nutrient (crude protein) and plant secondary metabolite (PSM unknown #1) covariates occurring in top models in both patch-scale and plant-scale analyses of Greater Sage-Grouse (*Centrocercus urophasianus*) winter habitat selection in south-central Idaho, USA. Intermediate values for samples in the non-browsed category suggest that observed patch-selection patterns were not artifacts of plant-scale effects.

were less precise for unknown #1 and α -pinene than for 1,8-cineole, as indicated by 95% confidence intervals slightly overlapping 1 (Table 1). The estimated classification accuracy for the top model was 80%. The odds of plant use increased by 102% for each 1% increase in crude protein, whereas the odds of plant use decreased by 18% for each 1 AUC/100 μ g increase in 1,8-cineole (Table 1, Fig. 2B).

Cross-scale correlation

Bivariate correlation coefficients did not indicate the presence of cross-scale correlations ($|r| < 0.7$). Similarly, no relationship was evident ($|r| < 0.7$) between the cumulative PSM values, found to be important at the habitat-type scale, and top covariates at the plant and patch scales. Plots of means partitioned by sample category for covariates shared among top models at the patch and plant scales (crude protein, unknown #1) indicated that non-browsed samples were intermediate in value relative to browsed and random samples (Fig. 3)

DISCUSSION

Results were largely consistent with our prediction that selected habitats would be characterized by high nutrient and low PSM concentrations. The sole exception occurred at the habitat-type scale, where nutrient concentrations were lower in the selected habitat type (black sagebrush). PSM differences at that scale may have been sufficiently extreme to negate the influence of

nutrients on habitat selection. Total monoterpene concentrations were 32% higher in Wyoming big sagebrush than in black sagebrush, whereas crude protein concentrations were only 10% higher (Fig. 1), suggesting that the increase in toxic consequences from high PSM concentrations in Wyoming big sagebrush may have outweighed the increase in nutritional benefit. Additionally, black sagebrush habitat was substantially lower in height than Wyoming big sagebrush habitat (Fig. 1), and the former may thus have provided lower quality thermal or escape cover. Again, the benefit of substantially lower PSMs in black sagebrush habitat may have outweighed any costs of increased thermal or predation risk. Alternatively, habitats characterized by lower shrub heights may have provided structural benefits for Sage-Grouse (e.g., increased ability to detect predators). Selection of low-height sagebrush habitat over sagebrush with greater cover potential has been observed in previous Sage-Grouse studies as well (Hagen et al. 2011), but associated phytochemistry has not been reported previously.

Habitat selection at the patch and plant scales was consistent with our prediction. Patches selected within black sagebrush habitat had higher nutrient concentrations and lower concentrations of individual PSMs (Table 1, Fig. 2). This suggests that use of patches by Sage-Grouse within a selected habitat type is nonuniform and is influenced by nutrients and PSMs. Unlike the habitat-type scale, however, total monoterpene concentrations within black sagebrush habitat did not differ between used (65.3 ± 1.88 AUC/100 μ g, mean \pm SE) and random (62.2 ± 2.06 AUC/100 μ g) patches. This is consistent with observations that sagebrush PSM profiles are more similar within taxa than among taxa (Kelsey et al. 1982). Our results suggest that concentrations of individual monoterpenes, rather than cumulative monoterpenes, influence patch selection within a habitat type. In other words, Sage-Grouse selected for the general PSM profile of black sagebrush (habitat-type selection), and then selected for more specific nutrient and PSM differences within that general profile (patch selection).

Similarly, individual plants selected within a given patch were higher in nutrient concentrations and lower in individual PSM concentrations. This suggests fine-scale habitat selection in which the use of plants within a patch is nonuniform and is influenced by nutrient and PSM characteristics. As with patch selection, total monoterpene concentrations were similar between browsed (63.7 ± 2.1 AUC/100 μ g, mean \pm SE) and non-browsed (66.9 ± 2.4 AUC/100 μ g) plants, but individual monoterpene concentrations, in concert with nutrients, appeared to affect plant selection.

An important consideration in multi-scale habitat selection studies is cross-scale correlation (Kristan 2006). Cross-scale correlations can yield inferences at one spatial scale that are actually attributable to effects at a different scale. Although bivariate correlations did

not indicate substantial cross-scale correlations, it is likely that inferences made at each spatial scale in our study lacked complete independence because we focused on the same phytochemical characteristics at all scales. In particular, because the patch effect was partially a function of plant-scale chemistry, patch-scale parameter estimates may simply have been an artifact of plant-scale effects for the covariates deemed important at both scales (crude protein, unknown #1). However, the fact that non-browsed samples were intermediate in both crude protein and unknown #1 relative to browsed and random samples (Fig. 3) suggests that selection was occurring for higher nutrients and lower PSMs at both spatial scales, and that patch-scale effects were not artifacts of cross-scale correlation. Similarly, the lack of substantial correlation between top covariates at the habitat-type scale and those at the smaller scales suggests that selection of habitat type was not solely an artifact of patch or plant selection.

These habitat-use patterns suggest that Sage-Grouse hierarchically selected habitat on the basis of multi-scale heterogeneity in phytochemistry. Within a landscape, Sage-Grouse selected a habitat type (coarse-scale selection) with substantially lower cumulative PSM concentrations than the alternative, despite lower nutrient concentrations and lower vegetation height. Within that habitat type, cumulative PSM concentrations did not vary, but Sage-Grouse selected patches (meso-scale selection) with higher nutrient concentrations and lower individual PSM concentrations. Within those patches, Sage-Grouse selected individual plants (fine-scale selection) with higher nutrient concentrations and lower concentrations of individual PSMs.

Multi-scale habitat selection by a vertebrate herbivore on the basis of phytochemistry has previously been documented only in marsupials within fenced reserves (Moore et al. 2010). Moore et al. (2010) found that koalas (*Phascolarctos cinereus*) in a fenced reserve were more likely to use patches with higher concentrations of crude protein and less likely to use patches with higher concentrations of a group of phenolic PSMs. These nutrient and PSM characteristics were also associated with koala use of individual trees within those patches (Moore et al. 2010). Our results were consistent with those of Moore et al. (2010) and provide the first evidence for hierarchical habitat selection on the basis of phytochemistry by a vertebrate herbivore outside of fenced reserves. Moreover, we documented habitat selection at a third scale (habitat type) that was not considered in previous studies, and provide the first evidence for the influence of phytochemistry on coarse-scale and meso-scale habitat selection by an avian herbivore.

We recommend that investigators routinely consider the functional importance of phytochemistry to avoid incomplete or inaccurate inferences in studies of herbivore habitat selection. This is particularly important for systems in which herbivores specialize on high-

PSM plants, as dietary constraints that limit use of landscapes may exacerbate the impacts of anthropogenic habitat changes. Specialist herbivore species that have suffered significant declines in abundance and available habitat, such as Sage-Grouse (Garton et al. 2011), may be particularly susceptible to environmental changes that decrease nutrient availability or increase PSMs (Lawler et al. 1997), and warrant prioritization for future research and conservation efforts.

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SUPPLEMENTAL MATERIAL

Appendix A

A photograph of diagnostic bite marks left by Greater Sage-Grouse (*Centrocercus urophasianus*) on sagebrush, *Artemisia* spp. ([Ecological Archives E094-025-A1](#)).

Appendix B

Supplementary information on the quantification of monoterpene concentrations in sagebrush (*Artemisia* spp.) samples, including methods, materials, and a table of gas chromatograph retention times ([Ecological Archives E094-025-A2](#)).

Appendix C

Methods and materials used to quantify total phenolic concentrations in sagebrush (*Artemisia* spp.) samples ([Ecological Archives E094-025-A3](#)).

Appendix D

Model-selection tables for hierarchical analysis of Greater Sage-Grouse (*Centrocercus urophasianus*) habitat selection at each of three spatial scales ([Ecological Archives E094-025-A4](#)).

Appendix E

A cross-tabulation of patch type by habitat type for analyzing the selection of sagebrush (*Artemisia* spp.) habitat type by Greater Sage-Grouse (*Centrocercus urophasianus*) ([Ecological Archives E094-025-A5](#)).

STUDY I, Job VII: Response of Greater Sage-Grouse to Wind Power Development in Idaho: Final Report

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ABSTRACT: The Idaho Department of Fish and Game (IDFG) has been studying this greater sage-grouse (*Centrocercus urophasianus*) population continuously since 2002 for multiple studies and formally initiated research on the impacts of wind power to greater sage-grouse beginning 1 July, 2011; commencing with the establishment of a funding agreement between IDFG and the Western Association of Fish & Wildlife Agencies (WAFWA) in conjunction with the wind farm developer. The research project involved a Before-After-Control-Impact (BACI) design utilizing both a treatment (Browns Bench) and control (Mary's Creek) area. The treatment area encompassed the proposed China Mountain Wind Farm Project. Funding from the Federal Aid for Wildlife Restoration was used for supervision of field crews and writing the final report. Seventeen sage-grouse (15 females, 2 males) were captured and fitted with solar-powered satellite platform terminal transmitter (PTT) during 2012. Battery-powered very high frequency (VHF) transmitters were placed on 58 sage-grouse (31 females, 27 males) and monitored for movements, survival, and nest success.

Survival was $83 \pm 16\%$ for females and $73 \pm 33\%$ for males on the treatment area (January – July, 2012). Female survival was $33 \pm 20\%$ and male was $51 \pm 17\%$ on the control area (February-October, 2012). Apparent nest success was 33% on the treatment area and 63% on the control area. Movements were similar to past research projects on both areas and grouse continued to use the proposed wind farm impact zone for seasonal habitat. No further work is being conducted on this project other than retrieval of PTT transmitters as mortalities occur.

STUDY I, Job VIII: Estimating Grass Height at Nest Initiation for Greater Sage-Grouse in Idaho

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ABSTRACT: Quality nesting habitat is important for increasing populations of greater sage-grouse (*Centrocercus urophasianus*). Past research has combined grass species and measured after nests hatched. My research focused on individual grass species at nests and random plots. I also calculated grass height at the beginning of incubation based on grass growth within 50 m of 100 nests actively being incubated. Grass structure was measured on 3 drupe height structures (residual, live, flower), number of flower stalks, and horizontal cover (effective height). Percent ground cover was also measured. Six grass species were the most abundant and occurred at a majority of the nests: Sandberg bluegrass (81% of nests, *Poa secunda*), bluebunch wheatgrass (49%, *Pseudoroegneria spicata*), bottlebrush squirreltail (47%, *Elymus elymoides*), cheatgrass (39%, *Bromus tectorum*), Idaho fescue (17%, *Festuca idahoensis*), and Indian ricegrass (16%, *Achnatherum hymenoides*). Cheatgrass residual height increased 8%, Idaho fescue increased 3% and the rest of the common grasses decreased 6-37% in residual height with Indian ricegrass declining the most. Nest sites had taller residual grass height for two of the four native bunchgrasses (bluebunch wheatgrass and Idaho fescue) than occurred at random. Bluebunch wheatgrass and Sandberg bluegrass had taller live grass height at nests than at random. Bluebunch wheatgrass had taller flower stalks whereas cheatgrass had shorter flower stalks at nests than at random. Nests had significantly more bluebunch wheatgrass ground cover (7.8%) than available at random (5.4%). Female greater sage-grouse might be selecting for sites with greater bluebunch wheatgrass because this species provides taller plant structure for concealment of nests. Future work includes developing variables from the dataset that allows for multivariate statistical analysis to determine if nest success is influenced by species specific habitat selection.

INTRODUCTION

Understanding what constitutes good nesting habitat for greater sage-grouse (*Centrocercus urophasianus*) is important for land managers so landscapes can be managed properly. Basic structure of nesting habitat has been well documented and summarized by Connelly et al. (2000) and Connelly et al. (2011) but all previous literature have measured habitat after nesting cessation, except for Hausleitner et al. (2005) who measured previously used nests to calculate cover changes during the following nesting season. Plant structure surrounding the nest, especially grasses, changes rapidly during the month between nest initiation and hatching. Greater sage-grouse nest initiation begins approximately 10 days after breeding (Autenrieth 1981). Egg laying requires 1.3 days/egg laid with an average of 7 eggs/nest (Patterson 1952) and incubation lasts 26 days (Pyrah 1954). Habitat at nest sites are typically measured after the female leaves to avoid abandonment or attracting predators. Coinciding measurements during incubation breaks requires an inordinate amount of vigilance and does not allow enough time for adequate vegetation sampling due to variability among females (Coates and Delehanty 2009) and human activity around nests could attract predators (Apa 1998). Measuring nest site vegetation after hatch may not reflect grass structure females were responding to during nest initiation and may not allow complete understanding of reasons for unsuccessful nests. The landscape surrounding the nest changes from dormant residual grasses produced during the previous year,

to lush vegetation as the growing season progresses. Factors that influence nest-site selection could involve dormant vegetal structure at the time of nest selection that may change by the end of incubation.

The biological hypothesis is successful nests have greater grass structure at nest initiation than unsuccessful nests and nests, in general, have greater grass height than available at random for the most common grass species present. Also, I hypothesized successful nests occur at sites with greater grass growth than unsuccessful nests.

OBJECTIVES

1. Determine changes (growth) in grass structure (height and horizontal cover) from early to end of incubation for successful vs. unsuccessful greater sage-grouse nests.
2. Estimate grass structure at nest initiation and compare between successful and unsuccessful nests.
3. Determine differences in grass structure between nests and habitat available at random for the most common grass species present.

STUDY AREAS

This research was conducted on 7 greater sage-grouse breeding populations during 2003-2005 (Fig. 1). The study areas are distributed throughout southern Idaho ranging in elevation from 1,600-2,400 m in a variety of shrub-steppe habitat types and range conditions and were previously described by Musil (2011). Because at least 12 habitat types (Hironaka et al. 1983) were present on the study areas and each area had multiple habitat types, habitat was instead categorized as sites dominated by dwarf sagebrush (little sagebrush [*Artemisia arbuscula*] and black sagebrush [*A. nova*] sagebrush), tall sagebrush (Wyoming big sagebrush [*A. tridentata wyomingensis*], mountain big sagebrush [*A. t. vaseyana*], and basin big sagebrush [*A. t. tridentata*]), three-tip sagebrush (*A. tripartita*), and mesic habitat found at higher precipitation zones with mixtures of tall sagebrush species, bitterbrush (*Purshia tridentata*), and berry producing shrubs (common chokecherry [*Prunus virginiana*], common serviceberry [*Amelanchier arborea*], and mountain snowberry [*Symphoricarpos oreophilus*]). Most (47%) of the study plots were in tall sagebrush habitat, 34% in dwarf sagebrush, 10% in threetip sagebrush sites, and 9% in mesic mixed shrub sites. Most common grass species occurring at nests include Sandberg bluegrass (*Poa secunda*, POSE), bluebunch wheatgrass (*Pseudoroegneria spicata*, PSSP), bottlebrush squirreltail (*Elymus elymoides*, ELEL), cheatgrass (*Bromus tectorum*, BRTE), Idaho fescue (*Festuca idahoensis*, FEID), and Indian ricegrass (*Achnatherum hymenoides*, ACHY). The study areas were on public and private land and are grazed in accordance with federal leases administered by the Bureau of Land Management (BLM) or the U. S. Forest Service (USFS).



Figure 1. Seven breeding populations of greater sage-grouse sampled for grass growth at nest sites in Idaho, 2003-2005. Populations are 1) Cow Creek, 2) Oreana/Big Springs/Shoofly, 3) Sheep Creek, 4) Browns Bench/Shoshone Basin, 5) Laidlaw Park, 6) Birch Creek, and 7) Little Lost Creek. Elevation is depicted by shading from low (dark) to high (white) elevations.

METHODS

Capture and marking

Greater sage-grouse females were captured by night lighting (Giesen et al. 1982, Wakkinen et al. 1992) on or near leks and fitted with 16.5g necklace-style radio transmitters (Riley and Fistler 1992) during 2002-2005. Nest locations of incubating greater sage-grouse were obtained from radio-marked females monitored for other ongoing studies. Females were determined to be nesting when they were either visually located on a nest or located on the same site 3 days in a row. Hand-held GPS units were used to re-locate nests as well as written descriptions of the site to avoid flushing females from nests during vegetation sampling.

Vegetation sampling

Measurements of grass structure by species were taken at a plot 30-50 m (ocular distance estimation) from incubating radio-marked females to determine growth phenology of the nesting habitat during 2003-2005. These targeted “near-nest” plots were located by ocular estimates of cover with similar elevation, aspect, shrub density, and shrub height to the nest. Measurements were taken within 1 week of initiation of incubation. The same sampling scheme for grass height measurements at nest sites (Musil 2011) was also conducted at near-nest plots and plots randomized within the available nesting habitat of the study area. While females were

incubating, only grass at the near-plot was measured to minimize human activity near the nest, shrubs and forbs were not measured. Center of 12 subplots were marked with wooden tongue depressors (Fig. 2A) at 1, 3, and 5 m from center of near-plot in 4, perpendicular directions with the first direction randomized. The closest grass, by species, at each of the 12 subplots was measured for drupe height, defined as the tallest naturally growing portion of the plant (Connelly et al. 2003), and marked with stick pins (Fig. 2B) so the same plant could be measured again at the end of incubation. Droop height of residual (previous season growth) and live (current season growth) excluding flower stalks, and tallest flower stalk (tallest of residual or live) for each grass species was measured separately. The number of flower stalks was also counted. Effective height was measured by placing a meter stick behind the plant and estimating the tallest height concealing $>1/2$ of the 2.5 cm wide meter stick. Effective height is considered the height of plant structure that effectively provides horizontal concealment cover (Musil 2011). Grass at near-plot plots, paired with nest sites, was measured again within 1 week after nests hatched or on the predicted hatch date for nests that were abandoned or depredated. Measurements at random plots (one for each nest when possible) were conducted within 1 week of the hatch date for at least one nest in the study area.



Figure 2. Methods used to mark near-plot plots during measurement of grass height while greater sage-grouse hens were actively incubating nests 30-50 m nearby. A) Wooden tongue depressor marks 3 m north of plot center with yellow stickpin marking closest grass species (arrow points to stickpin). B) Yellow stickpin marking grass to be measured again after incubation is completed.

Ground cover was measured with a modified Daubenmire (1959) frame but only at nest and random sites, not near-plot plots. Cover categories were modified to include more sensitivity for lower cover values for the following cover classes: 1 (0-1%), 2 (2-5%), 3 (6-25%), 4 (26-50%), 5 (51-75%), and 6 (76-100%). The modification to the ground cover method was to double the area observed for each plot (40 x 50 cm rather than 20 x 50 cm) to increase the area measured

because shrub steppe habitat is less dense than what was originally developed by Daubenmire (1959).

Statistical analysis

Kruskal-Wallis (K-W) non-parametric one-way analysis of variance was used to compare differences in means (R [Version 2.15.3, www.R-project.org, accessed March 2013]) for multiple variables and Wilcoxon rank sum tests to conduct multiple comparisons when overall K-W test was significant at the 0.05 level of significance. Estimates of means were bound by 95% CI. Paired observations (nest vs. near-nest) were compared using sign test (Conover 1980).

RESULTS

We measured nesting habitat at 100 paired nest/near-nest plots (64% of all nests sampled) with 71% of these nests incubated by adults and 37% successfully hatching (72% of successful nests were incubated by adult females) during 2003-2005. Near-nest plots averaged 41.9 ± 2.8 m from nests and distances were not different between successful (44.2 ± 5.1 m) and unsuccessful (45.0 ± 3.6 m) nests ($P = 0.60$). Number of days elapsed between when near-nests were first measured and re-measured after nesting averaged 25.3 ± 1.1 days and were not different between successful (24.4 ± 1.9 days) and unsuccessful (25.9 ± 1.3 days) nests ($P = 0.22$). An additional 56 nests without paired near-nest plots and 138 random plots were also sampled. Grass that was grazed was excluded from the analysis. Measurements of grass structure at near-nests took an estimated 15-20 minutes to minimize disturbance of the incubating hen or attract predators.

Six grass species were the most common among the nest sites and were used for comparing changes in plant structure during the nesting season (Fig. 3). Sandberg bluegrass was the most common grass species occurring at 81% of the nest and near-nest paired plots, followed by bluebunch wheatgrass (49%), bottlebrush squirreltail (47%), cheatgrass (39%), Idaho fescue (17%), and Indian ricegrass (16%). Most of the nest plots (37%) had at least 3 of the common grasses present while most of the near-nest plots (39%) had at least 2 of the common grass species present (Fig. 4). No plots had all 6 species present and 1 nest plot had none of the common grasses present while all the near-nest plots had at least 1 of the common grass species present.

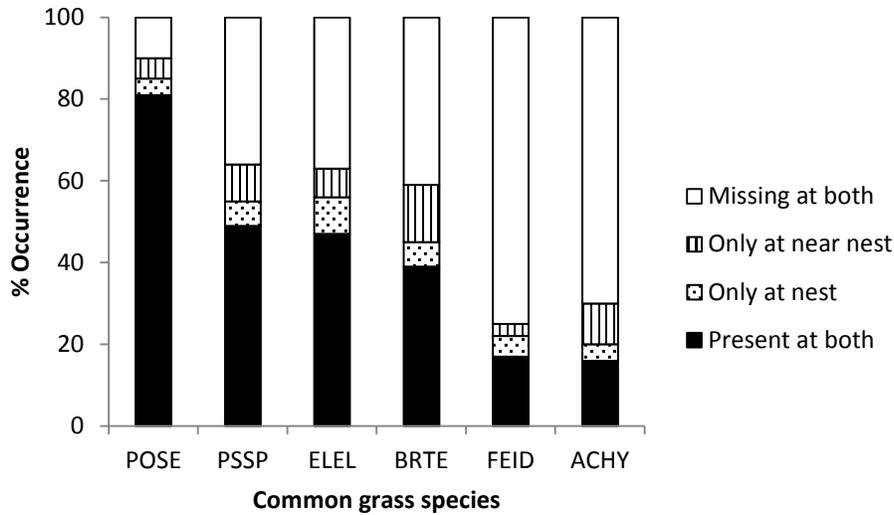


Figure 3. Grass species occurring at greater sage-grouse nest and near-nest paired plots (n = 100) in Idaho, 2003-2005. Grass species mnemonic: POSE = Sandberg bluegrass, PSSP = bluebunch wheatgrass, ELEL = bottlebrush squirreltail, BRTE = cheatgrass, FEID = Idaho fescue, and ACHY = Indian ricegrass.

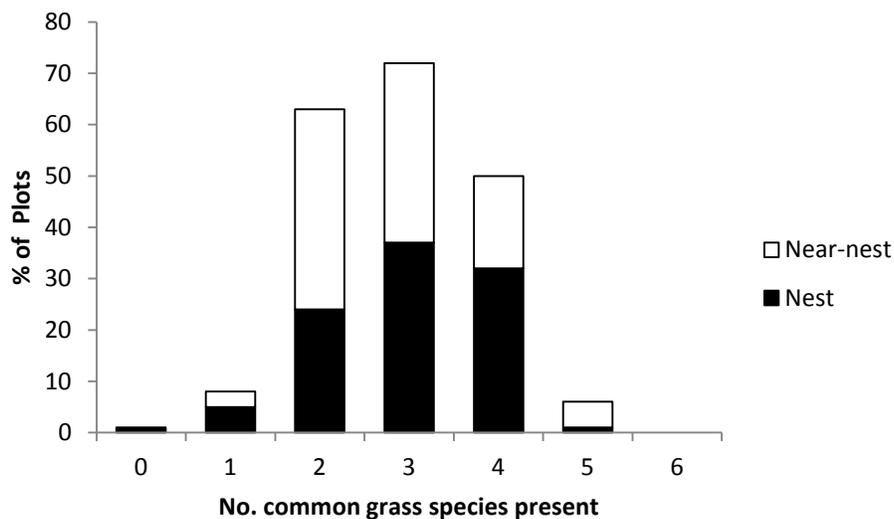


Figure 4. Percent of 100 paired plots where 6 of the most common grass species were present at greater sage-grouse nests and near-nests in Idaho, 2003-2005.

Four of the 6 species had declines in residual grass height from early incubation to after hatch date while 2 grasses increased in residual height (Table 1). Cheatgrass had significantly greater percent change (increase) in residual grass height than Indian ricegrass but was not different from the other grasses. Cheatgrass tended to have the highest percent increase in live and flower height along with increases in numbers of flower stalks but was statistically similar to growth changes by Indian ricegrass for these structures.

Table 1. Percent change (SE) in drupe height of plant structure for common grass species occurring near greater sage-grouse nest sites and measured during early incubation and after nest hatch date in Idaho, 2003-2005.

Grass ^a	n ^b	Residual	Live	Flower	No. flower stalks	Effective
POSE	85	-0.06 (-0.05) AB ^c	-0.02 (0.03) A	0.47 (0.05) A	0.22 (0.07) A	0.09 (0.02) AB
PSSP	55	-0.13 (0.05) B	0.29 (0.03) B	0.25 (0.05) B	0.16 (0.09) A	0.17 (0.04) A
ELEL	56	-0.08 (0.06) AB	0.17 (0.03) C	0.39 (0.06) AB	0.30 (0.08) A	0.12 (0.05) AB
BRTE	46	0.08 (0.04) A	0.27 (0.11) B	0.73 (0.06) C	0.72 (0.06) B	0.15 (0.05) B
FEID	22	0.03 (0.07) AB	0.23 (0.04) BC	0.35 (0.09) AB	0.23 (0.10) A	0.16 (0.07) AB
ACHY	20	-0.37 (0.17) B	0.04 (0.11) AC	0.45 (0.1) ABC	0.37 (0.11) A	0.11 (0.10) AB

^a POSE = Sandberg bluegrass, PSSP = bluebunch wheatgrass, ELEL = bottlebrush squirreltail, BRTE = cheatgrass, FEID = Idaho fescue, ACHY = Indian ricegrass.

^b Number of nest plots where grass species occurred.

^c Grasses with common letters within same plant structure (column) are not different at 0.05 level of significance.

Grass growth (% change from early to late incubation) was similar between successful and unsuccessful nests ($P>0.06$). Change in residual height of bottlebrush squirrel-tail ($P=0.082$) and number of Sandberg bluegrass flower stalks ($P=0.067$) were the only species and structures that neared a 0.05 level of significance, the rest of the comparisons had $P>0.24$. Grass height was similar ($P>0.05$), when measured at the end of incubation, between near-nest plots and the paired nest sites for the 6 common grass species except for height of residual Sandberg bluegrass was statistically taller ($P=0.03$) at nest sites (4.1 ± 0.6 cm) than near-nests (3.9 ± 0.7 cm) and residual height of bottlebrush squirreltail was taller ($P=0.009$) at nests (9.5 ± 1.1 cm) than at near-nests (8.1 ± 1.3 cm).

Comparing all 156 nests and 138 random plots, taller residual grass height occurred at nest sites than available at random for bluebunch wheatgrass and Idaho fescue (Table 2). Live grass height at nests was taller for bluebunch wheatgrass and Sandberg bluegrass than available at random. Bluebunch wheatgrass flower height was taller at nests than random but cheatgrass flowers were taller and had more flower stalks at random than nest plots. There were no differences in effective cover height by species. Only bluebunch wheatgrass ground cover was greater at nests than available at random for the 6 most common grass species (Table 3).

Table 2. Average drupe height of grass at time of hatch for greater sage-grouse and available at random in Idaho, 2003-2005.

Grass ^a	Residual			Live			Flower			No. Flower Stalks			Effective		
	Nest	Random	<i>P</i> ^b	Nest	Random	<i>P</i>	Nest	Random	<i>P</i>	Nest	Random	<i>P</i>	Nest	Random	<i>P</i>
POSE	4.2	4.5	0.946	8.2	7.8	0.008	23.7	24.9	0.611	4.8	4.9	0.865	4.2	4.1	0.699
PSSP	19.9	14.9	0.002	29.0	23.7	0.001	37.2	27.7	0.008	7.0	6.9	0.370	14.5	12.1	0.170
ELEL	10.8	10.4	0.276	20.3	19.1	0.350	22.5	21.8	0.669	6.5	6.9	0.668	10.4	11.0	0.522
BRTE	0.9	0.7	0.899	7.2	7.3	0.715	11.5	14.3	0.002	1.4	1.6	0.003	1.8	1.3	0.106
FEID	12.5	8.2	0.009	20.7	19.8	0.641	21.1	22.5	0.853	3.9	2.8	0.337	11.9	12.0	0.867
ACHY	11.0	9.0	0.232	20.5	18.1	0.394	16.1	15.7	0.734	6.0	2.2	0.811	6.9	7.0	0.352

^a POSE = Sandberg bluegrass, PSSP = bluebunch wheatgrass, ELEL = bottlebrush squirreltail, BRTE = cheatgrass, FEID = Idaho fescue, ACHY = Indian ricegrass,

^b Kruskal-Wallis nonparametric analysis of variance test between nests and random plots within grass structure.

Table 3. Percent grass ground cover (SE) at greater sage-grouse nests compared with random plots in Idaho, 2003-2005.

Grass	Nests	Random	<i>P</i> ^a
Bluebunch wheatgrass	7.8 (0.8)	5.4 (0.7)	0.018
Idaho fescue	12.8 (2.1)	12.3 (1.8)	0.982
Sandberg bluegrass	7.5 (0.5)	8.6 (0.7)	0.250
Cheatgrass	5.2 (0.8)	6.7 (1.2)	0.484
Bottlebrush squirreltail	2.2 (0.3)	2.4 (0.3)	0.579
Indian ricegrass	1.1 (0.2)	1.2 (0.3)	0.553

^a Kruskal-Wallis nonparametric analysis of variance test between nests and random plots (within row) of percent grass cover by species.

By using the percent change in grass height measured at near-nests, I estimated grass height early in incubation for the paired nests (Table 4). Residual height decreased significantly only for bluebunch wheatgrass. Live grass height increased for four of the six species whereas all species had significantly greater flower heights at the end of the incubation than at onset. The number of flower stalks increased for four of the six species and effective height increased for three of the six grasses.

Table 4. Grass drupe height at greater sage-grouse nests after hatch (late) and estimated for height in early incubation in Idaho, 2003-2005.

Grass ^a	Residual			Live			Flower			No. flower stalks			Effective		
	Early	Late	<i>P</i> ^b	Early	Late	<i>P</i>	Early	Late	<i>P</i>	Early	Late	<i>P</i>	Early	Late	<i>P</i>
POSE	4.2	4.2	0.777	8.7	8.5	0.973	9.7	20.5	<0.001	2.7	2.9	0.037	4.2	4.6	<0.001
PSSP	22.9	20.8	0.003	20.2	28.3	<0.001	16.1	31.6	<0.001	5.7	3.6	0.003	12.9	16.2	<0.001
ELEL	8.3	9.5	0.258	15.8	19.8	<0.001	9.8	19.6	<0.001	3.3	4.8	0.055	8.4	10.4	<0.001
BRTE	0.7	1.3	0.142	5.0	8.2	<0.001	2.2	9.6	<0.001	0.2	0.8	<0.001	0.3	0.4	0.271
FEID	12.0	12.3	0.230	15.9	20.5	<0.001	4.6	13.1	0.036	0.4	0.7	0.058	9.6	10.9	0.049
ACHY	15.7	11.5	0.078	18.8	20.2	0.062	4.7	14.9	0.004	0.1	4.3	0.002	6.5	7.1	0.103

^a POSE = Sandberg bluegrass, PSSP = bluebunch wheatgrass, ELEL = bottlebrush squirreltail, BRTE = cheatgrass, FEID = Idaho fescue, ACHY = Indian ricegrass

^b Nonparametric sign test between paired early and late measurements.

Grass height was similar between nests and random plots for three (Indian ricegrass, Sandberg bluegrass, and bottlebrush squirreltail) of the six common species at the three distances measured from the center of the plot. Nests had taller residual (20.3 ± 3.3 cm) bluebunch wheatgrass than available at random (12.7 ± 2.6 cm) at 1 m from the center of the nest or random plot ($P < 0.001$). Nests also had taller live bluebunch wheatgrass at nests (1 m = 29.1 ± 3.0 cm, 3 m = 27.4 ± 2.6 cm) than at random plots (1 m = 23.9 ± 2.4 , 3 m = 23.4 ± 2.5 cm [$P = 0.006$, $P = 0.011$, respectively]). Nests had consistently shorter flowers and effective height as well as fewer number of flower stalks for cheatgrass than random sites at all three distances from the center of the plot. Residual Idaho fescue was taller at 1 and 5 m from centers of nests (17.2 ± 6.5 cm, 11.0 ± 1.9 cm) than at random plots (7.2 ± 2.3 cm, 7.1 ± 2.5 cm [$P = 0.004$, $P = 0.0240$, respectively]).

DISCUSSION

We did not appear to influence nest success by measuring grass height near nests during early incubation. Our activities did not cause females to flush from nests or abandon. It is not known how close measurements could be made without influencing incubation but the closest near-nest plot was 19 m from the nest and this nest successfully hatched as did 2 other nests with near-nest plots within 30 m of the nest.

Six grass species were the most common among the nest sites; 5 native bunch grasses and 1 exotic annual. Because cheatgrass is an annual grass and therefore measurements are not necessarily made on the exact same plant, it was difficult to determine residual grass between previous season's material and from current year's structure already reaching dormancy. Therefore, increases in residual cheatgrass height are likely not a true increase but an error in measuring a different plant. Cheatgrass takes advantage of early moisture, a characteristic of cool season annuals, so it is not surprising it tends to show the most change in growth relative to its own size. Sandberg bluegrass is similar to cheatgrass in that it likely reaches maximum height of basal blades prior to nest initiation but increases height of flower stalks during incubation. The increase in residual height for Idaho fescue and decline in live height for Sandberg bluegrass is likely not significant due to poor sample size and high variability in the data, respectively.

Despite attempting to pick near-nest sites with similar sagebrush cover, aspect, and slope, the near-nest sites did not have complete consistency in occurrence of grass species compared to the paired nest site. Our ocular estimate for the location of near-nests was sufficient, though, to provide similar grass heights when paired with the nearby nests. Grasses at the paired plots were similar in height at the end of incubation and therefore the near-nest plots likely represented the growth rates at nests to accurately estimate grass height early in incubation. Grass growth was similar between successful and unsuccessful nests, which negated our theory that successful nests are on sites with greater growth thereby providing more cover earlier than at unsuccessful nests. Growth likely works in concert with other factors to influence nest success but is not significant alone.

Ground cover and height of all grass species combined were not significant variables when describing nests throughout southern Idaho in a multivariate analysis of this same dataset, but shrub height, shrub density, and horizontal cover were important variables (Musil 2011).

Individual species were not considered in that analysis because too many sites had inconsistent species for multivariate analysis.

Hausleitner et al. (2005) found a 2% increase in grass ground cover from nest initiation to hatch and grass height increased < 6 cm but did not compare among species or provide percent change of height for comparisons with our study. I found grass growth and deterioration of residual structure varied among grass species.

Female greater sage-grouse may be selecting sites with more bluebunch wheatgrass cover than available at random because it provides taller concealment cover than other species. Bluebunch wheatgrass tends to be one of the tallest of the common shrub-steppe grass species in southern Idaho and provides some of the greatest horizontal cover (Musil 2011). It also appears females are choosing nest sites with greater height of grasses, especially bluebunch wheatgrass, close to the nest, than what is available at random. This is consistent with results in other studies (Wakkinen 1990, Connelly et al. 1991, Gregg et al. 1994, DeLong et al. 1995, Connelly et al. 2000, Holleran et al. 2005, Connelly et al. 2011) but these studies combined results from multiple grass species. Combining grass species reduces the influence of species uniqueness for understanding habitat selection by nesting greater sage-grouse.

MANAGEMENT IMPLICATIONS

We did not measure grass growth at random plots so we cannot determine if female greater sage-grouse are selecting nest sites with greater cover at nest initiation than available at random, but it does appear that abundant and robust native bunch grasses, especially bluebunch wheatgrass, are important to females selecting nest sites when measured at the time of hatch. Efforts to maintain healthy and abundant native bunch grasses are consistent with recommendations in the greater sage-grouse guidelines (Connelly et al. 2000). Adequate concealment cover surrounding nests allows females to take incubation breaks without attracting the attention of predators and thereby increase the probability of hatching a nest. Nest success is a critical factor influencing greater sage-grouse population dynamics and any management activities that negatively impact nest success should be avoided to support healthy greater sage-grouse populations.

FUTURE WORK

I will be creating a variable or series of variables that encompass species diversity, origin (native vs. non-native), growth type (annual vs. perennial), and habitat type in order to conduct multivariate statistical analysis for this data set. Because not all grass species are present at all nest plots, multivariate analysis cannot be conducted because missing data for the missing species causes the entire plot to be discarded. Concluding this analysis will determine if nest success is influenced by species specific habitat selection.

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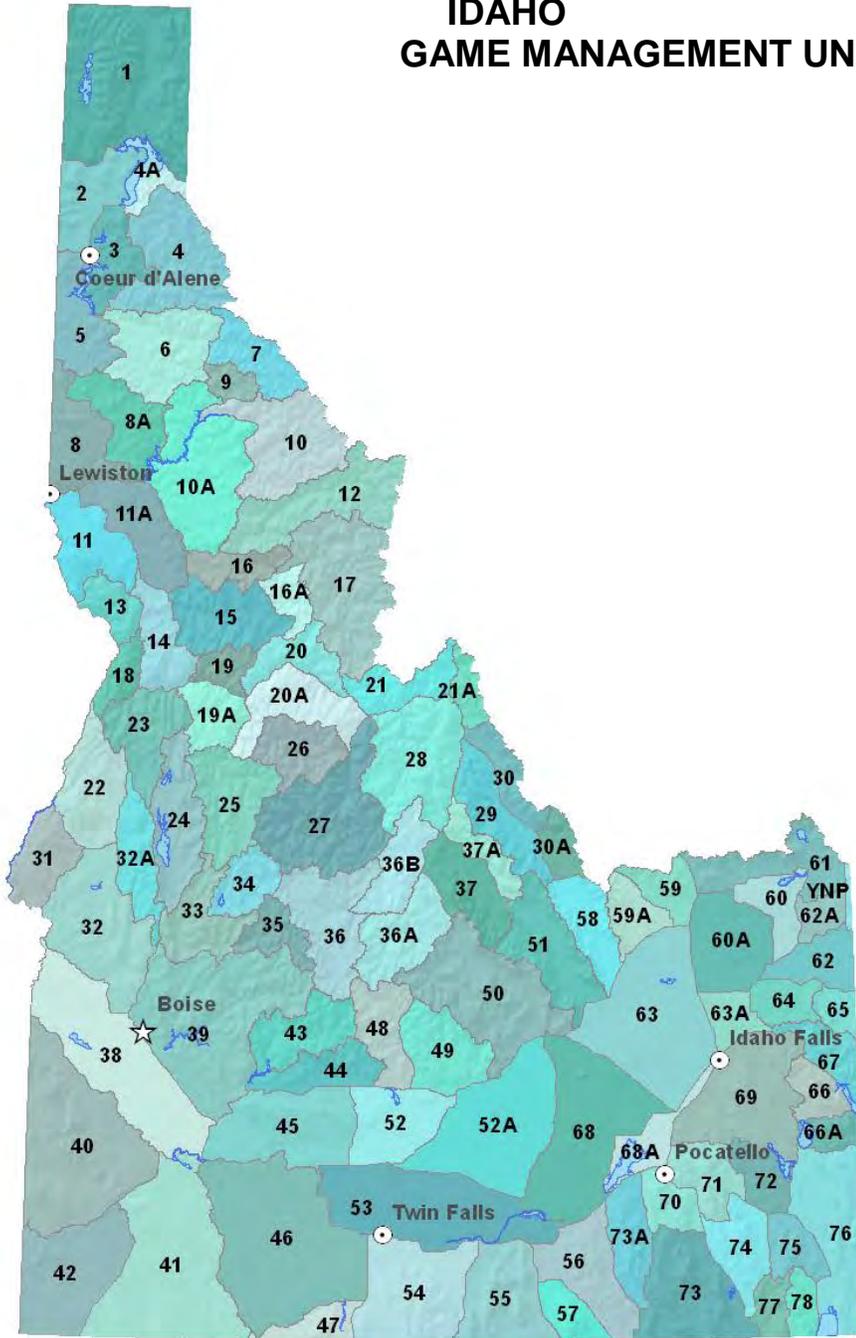
STUDY II: PHEASANT ECOLOGY AND MANAGEMENT

Territorial Male Pheasant Density Response to Habitat Changes

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83338, USA

Progress during the performance period was limited to data editing, compilation, and accuracy verification.

IDAHO GAME MANAGEMENT UNITS



FEDERAL AID IN WILDLIFE RESTORATION

The Federal Aid in Wildlife Restoration Program consists of funds from a 10% to 11% manufacturer's excise tax collected from the sale of handguns, sporting rifles, shotguns, ammunition, and archery equipment. The Federal Aid program then allots the funds back to states through a formula based on each state's geographic area and the number of paid hunting license holders in the state. The Idaho Department of Fish and Game uses the funds to help restore, conserve, manage, and enhance wild birds and mammals for the public benefit. These funds are also used to educate hunters to develop the skills, knowledge, and attitudes necessary to be responsible, ethical hunters. Seventy-five percent of the funds for this project are from Federal Aid. The other 25% comes from license-generated funds.

