

Design for Mosquito Sampling in Relation to Sage-Grouse Habitat Use

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Introduction

In 2003, West Nile virus (WNV) spread into the eastern portion of the current range of greater sage-grouse (*Centrocercus urophasianus*). Late summer survival in four affected populations in Alberta, Montana, and northern and southern Wyoming, declined by approximately 25% compared to both pre-WNV infection survival and compared to a uninfected control population in 2003 (Naugle et al. 2004). Limited mosquito sampling conducted in the late-summer in northern Wyoming indicated that *Culex tarsalis*, a common prairie mosquito, was a competent vector of WNV (Naugle et al. 2004). Large sage-grouse populations, over time, may evolve to develop population level resistance, however, serum from 112 sage-grouse collected after the outbreak in 2003 indicated that sage-grouse have extremely low immunity to the virus, if any at all (Naugle et al. 2004). This raises immediate concerns over how to manage small endangered populations of sage-grouse, like those found in southeastern Alberta and southwestern Saskatchewan (Aldridge and Brigham 2003). Thus, we propose that controlling mosquito populations to reduce the chance of sage-grouse being exposed to WNV may be a viable management option. Here, we propose an experimental design aimed at 1) developing treatments to reduce mosquito populations within the range of greater sage-grouse in Alberta, 2) testing the effectiveness of those treatments at reducing mosquito abundance, 3) testing if treatments reduce the prevalence of WNV in mosquitoes, and 4) assessing the effectiveness of reduced mosquito abundance at ultimately reducing sage-grouse mortality due to WNV.

Sampling Design

To test the effectiveness of larvicide to control mosquitoes, and reduce the risk of the infection of sage-grouse with WNV, we had to first identify areas that were 1) likely to contain standing water and thus, abundances of mosquito larvae, and 2) likely to contain sage-grouse during the summer. Thus, we created models that best identify the co-occurrence of Sage-Grouse 'habitat', based on a brood habitat resource selection function model (RSF, Manly et al. 2002) predicting a high probability of sage-grouse occurrence, and a model that predicted a high probability standing water (mosquito habitat).

To identify standing water, we estimated an index for wetness referred to as the compound topographic index (CTI; Moore et al. 1993, Geissler et al. 1995). We used an ArcInfo algorithm (Rho 2002) and a digital elevation model to calculate CTI, which basically 'fills' the landscape with water, identifying where water will pool, creating a moisture index (Figure 1). We used existing standing water bodies (Alberta provincial base features data; 1:20,000 scale) to determine which CTI values identified known standing water, acting to anchor our CTI index. The average CTI value at water bodies was 13.4. We subtracted one standard deviation (2.7) from the mean CTI value, and to be conservative, considered CTI moisture index values ≥ 10.0 to indicate a potential standing water source indicative of suitable mosquito breeding habitat. We then classified each cell as 1 (mosquito habitat) or 0 (not mosquito habitat).

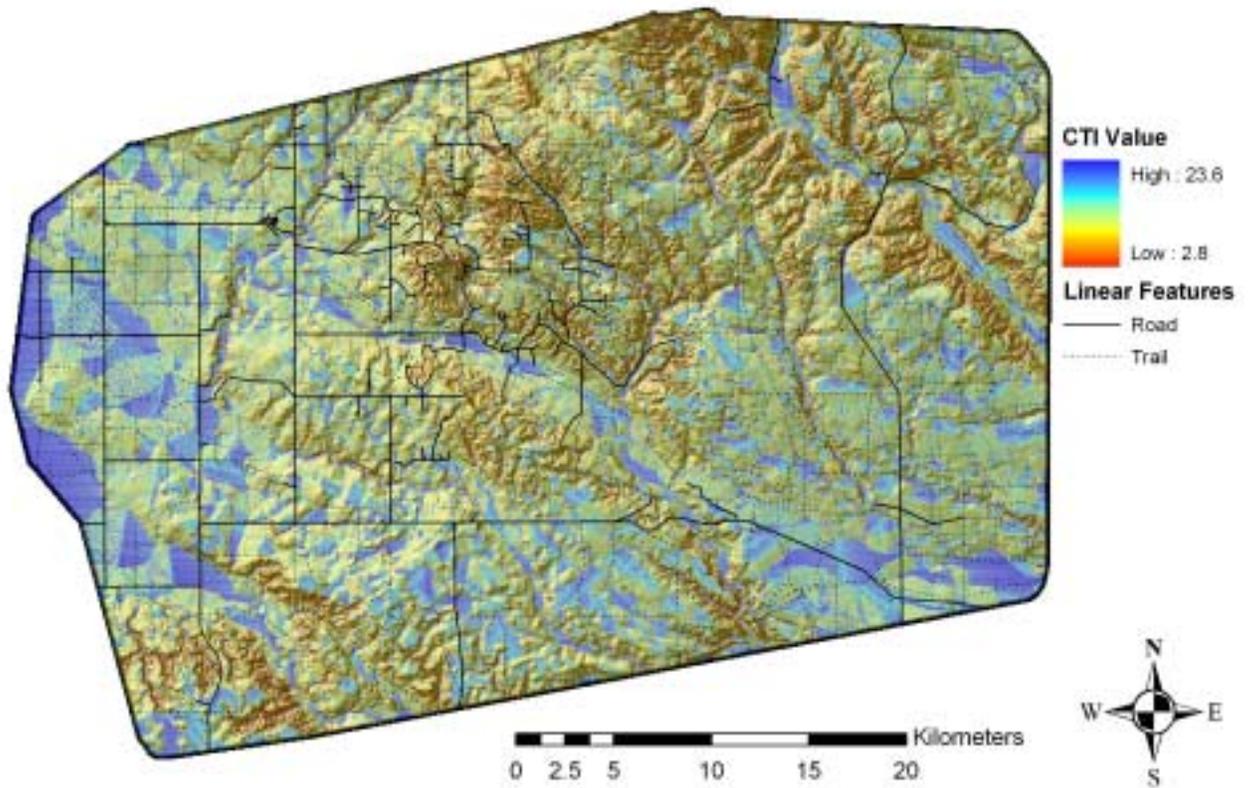


Figure 1. Compound topographic index of wetness over the sage-grouse brood habitat study area. High index values (blue) indicate increased moisture values.

We used an RSF model previously developed by C.L. Aldridge (unpublished data) for sage-grouse within this study area for data collected from 1998-2003. This RSF model has been shown to accurately predict sage-grouse brood rearing habitat (Figures 2, 3) and took the general log-linear form:

$$w(x) = \exp(\beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \dots + \beta_n x_n)$$

where $w(x)$ is the resource selection function (relative probability of occurrence) and each β_i is the selection coefficient for the particular habitat variable (x) of interest (see Table 1 for variable descriptions).

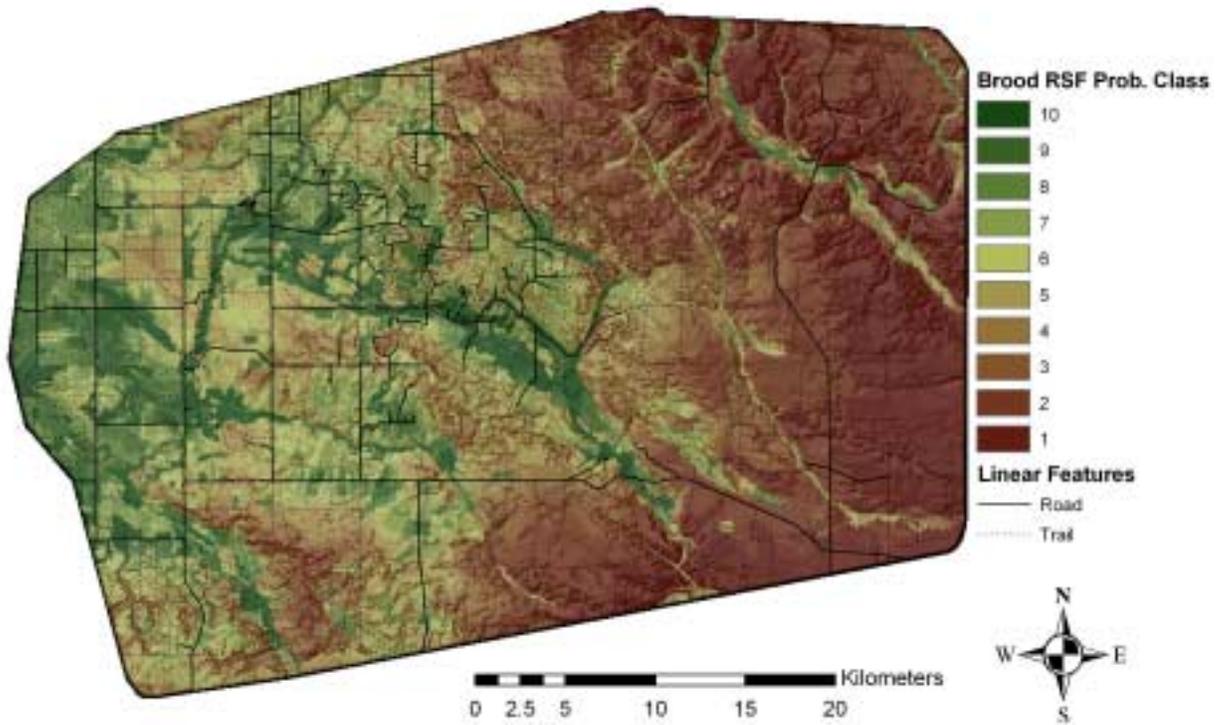


Figure 2. Brood resource selection function (RSF) model indicating the probability of habitat being used by brooding female sage-grouse during July and August. RSF scores scaled and binned into 10 classes.

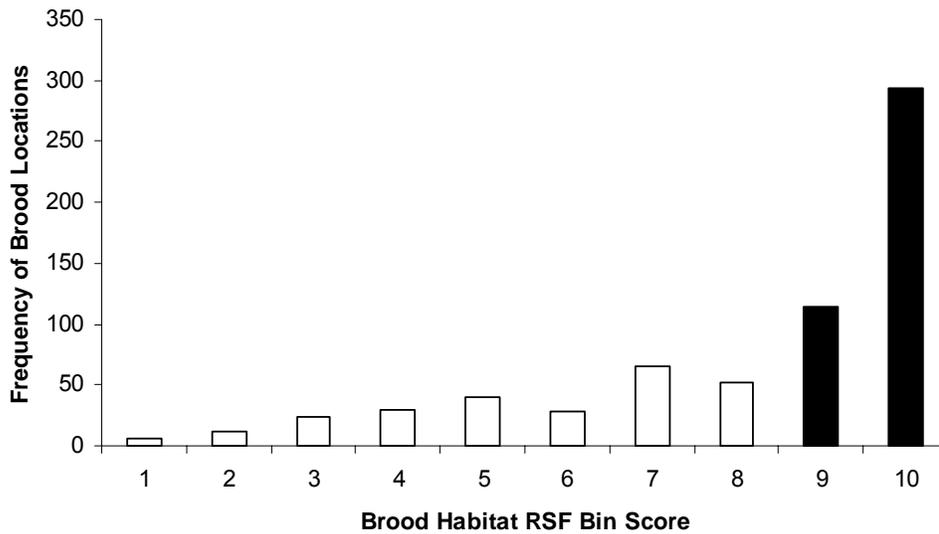


Figure 3. Within sample validation for the best brood habitat RSF model. Bins 9 and 10 contained 61% (408/664) of sage-grouse brood telemetry relocations. RSF Bins 9 and 10 (black bars) were used as a conservative cut off considered sage-grouse brood occurrence.

The best model based on Akaike Information Criteria (AIC, Anderson et al. 2000) from this approach was as follows:

$$w(x) = \exp[0.08(\text{Dist-Imped}) + 0.47(\text{Dist-Trail}) - 0.14(\text{Dist-Road}) - 0.2(\text{Dist-Well}) + 0.02(\text{Wet}) + 0.01(\text{Green}) - 1.45(\text{Shrub}) - 0.37(\text{Habitat}) + 0.03(\text{SBCover}) - 0.06(\text{Slope}) - 0.001(\text{DEM}) + 0.94(\text{SBPatch})]$$

Table 1. Description of the variables included in the best resource selection function model (based on AIC) predicting sage-grouse brood habitat in southern Alberta.

Variable	Description
Dist-Imped	Distance from nearest water impediment (dugout/dam/well)
Dist-Trail	Distance from the nearest trail
Dist-Road	Distance from the nearest road
Dist-Well	Distance from the nearest oil well site
Wet	Wetness index as derived from a satellite image
Green	Greenness index as derived from a satellite image
Shrub	Shrub habitat (typically along riparian areas, 1 or 0)
Habitat	Range ecosite potentials identified as important to sage-grouse (1 or 0)
SBCover	Sagebrush cover expressed as a percentage
Slope	Slope as derived from a digital elevation model
DEM	Elevation as derived from a digital elevation model
SBPatch	Patchily distributed sagebrush (1), as opposed to uniform, absence, or sparse (0)

Within-sample validation (Figure 3) on area-adjusted RSF bin scores (Boyce et al. 2002) indicated that this model accurately predicted sage-grouse brood habitat (increasing occurrence of brood locations in higher RSF bins; $r_s = 0.95$). We considered RSF Bins 9 and 10 to be a conservative cut off indicating a high probability of sage-grouse use (Figure 3). Thus, this model (based on 1998-2003 data) should be a suitable predictor of sage-grouse occurrence on the landscape during the 2004 WNV season (June through August).

Combining wet areas of CTI values ≥ 10 (Figure 3a) with areas of high probability (RSF bins are ≥ 9) of summer sage-grouse occurrence (Figure 2, 3b) we identified the co-occurrence of sage-grouse and mosquitoes within the study area (Figure

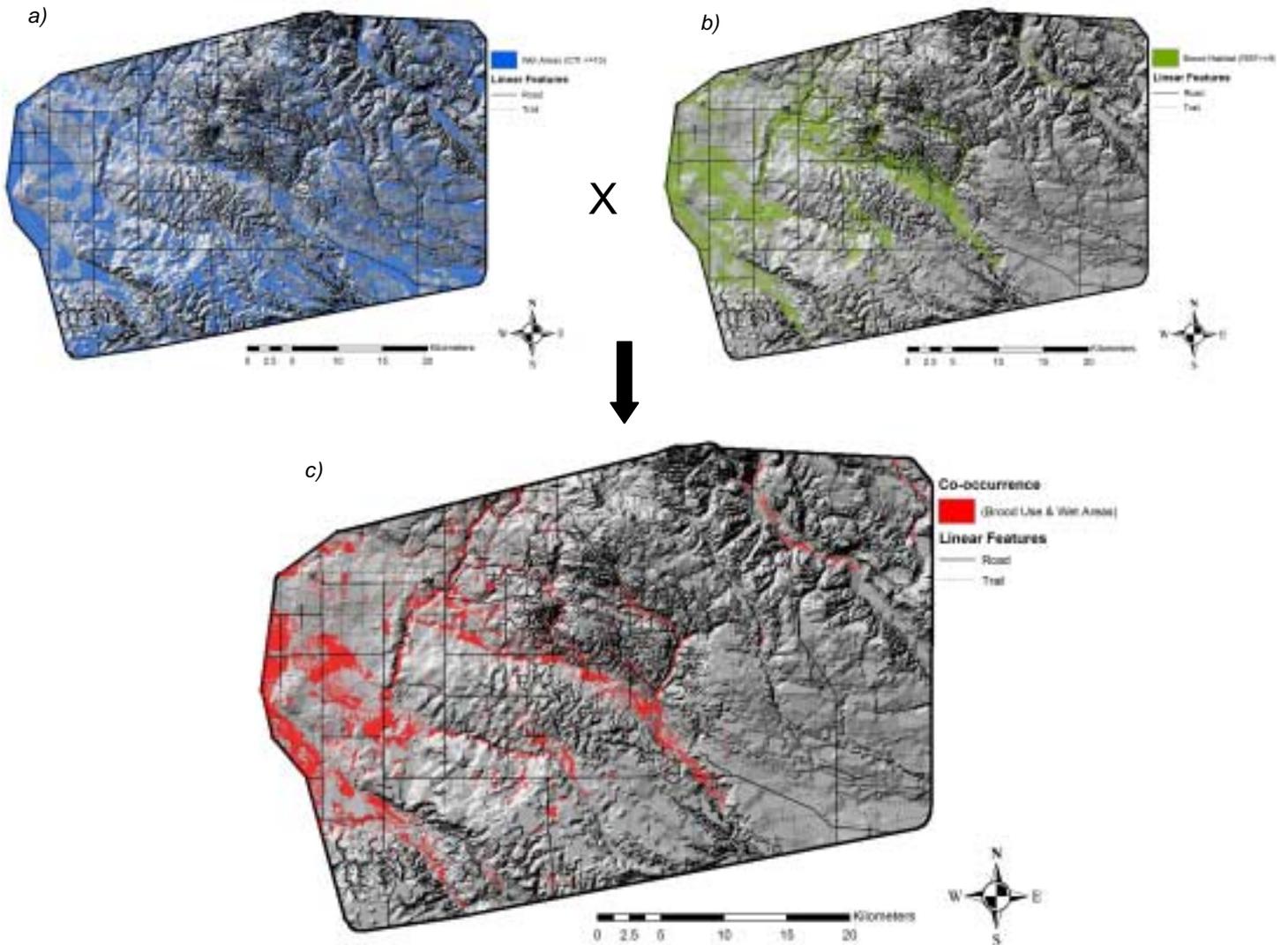


Figure 4. The co-occurrence (map c - red) of wet areas (CTI values ≥ 10 ; map a - blue) indicating a high probability of mosquito breeding habitat, and sage-grouse summer habitat use, as indicated by high brood habitat RSF probability scores (RSF bin score ≥ 9 ; map b - green).

3c). However, given that *Culex tarsalis*, the principal mosquito vector of West Nile virus in southern Alberta, can travel *ca* 5 km from standing water used as breeding/hatching areas, traps could be drawing in mosquitoes from up to 5 km away. Thus, co-occurrence models should be based on a moving window with a radius equivalent to the sampled

area (i.e. a 5-km radius of trapping sites). Given that time and financial constraints limited us to mosquito sampling using 4 traps, we decided to place two traps at a single treatment area (treated with a mosquito larvicide) and two traps at a single control area (no treatment). At each sample area, the two traps will be spaced 1-km apart, or 500 from the centroid of the chosen sampling area. Thus we would conceivably be sampling mosquitoes within a 6-km radius around the sample area centroid (5-km around each trap).

We estimated the proportion of area within a 6 km radius of every 30 m pixel that contained the co-occurrence (red areas in Figure 4c) of sage-grouse and mosquito habitat using a 6 km moving window (see Figure 5).

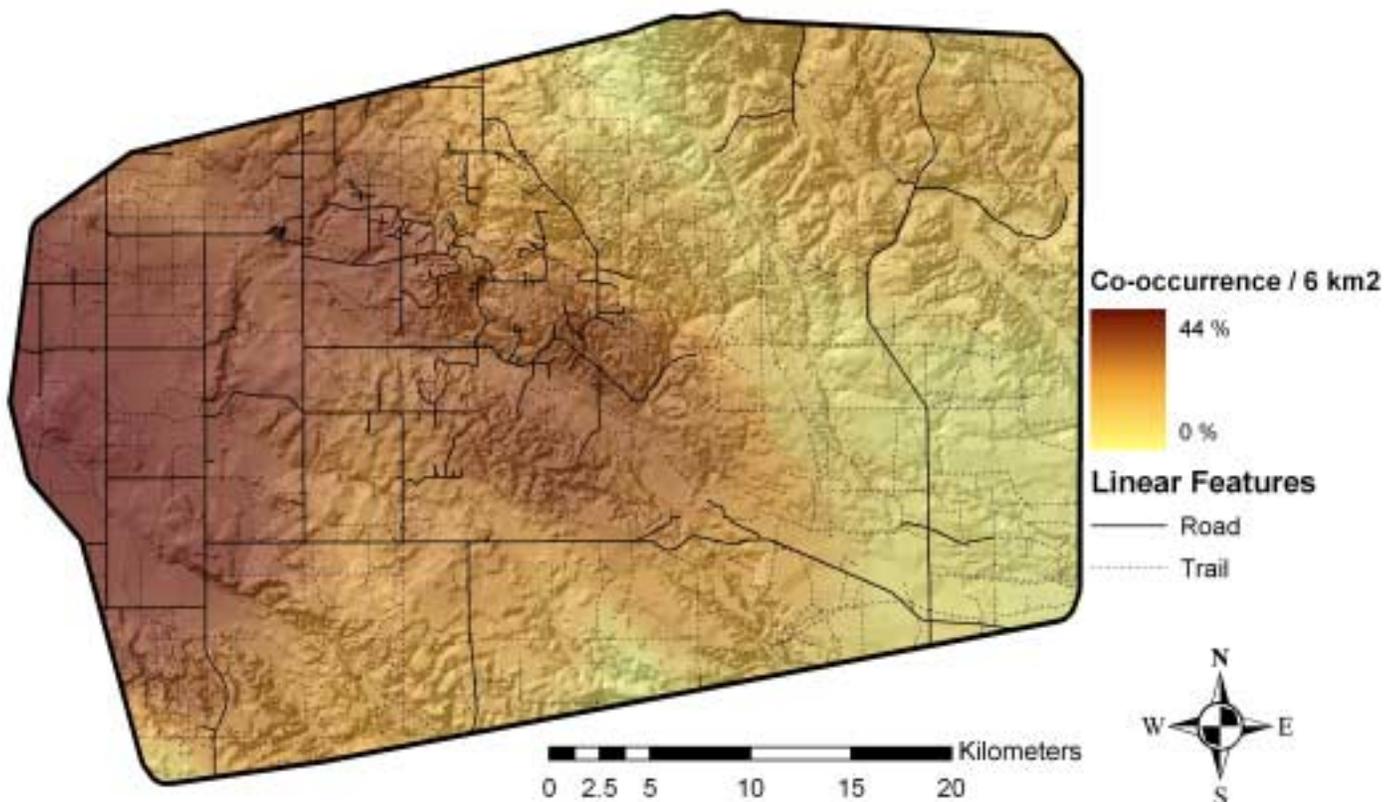


Figure 5. The proportion of habitat within a 6 km radius of each pixel on the landscape where both sage-grouse and mosquitoes are predicted to co-occur.

From this metric, for each 10 m cell on the landscape, we calculated the proportion of the 6 km² area around each 10 m pixel on the landscape (6 km moving window) that contained the co-occurrence of sage-grouse and mosquito habitat (Figure 5). The highest proportion of any 6 km² window with the co-occurrence of both sage-grouse use mosquitoes was 44%, but most cells were below 10% (Figure 6). Thus, we highlight the top fifth (20%), and top third (30%) of sample areas with co-occurrence, which corresponds to 20.4% and 9.5% of the cells within the area considered as co-occurrence, respectively (Figure 6). We considered only sample areas that had at least 9.5% of the sample area comprised of co-occurrence (red highlight in Figure 6) to be suitable potential sites for centering our two mosquito sampling areas.

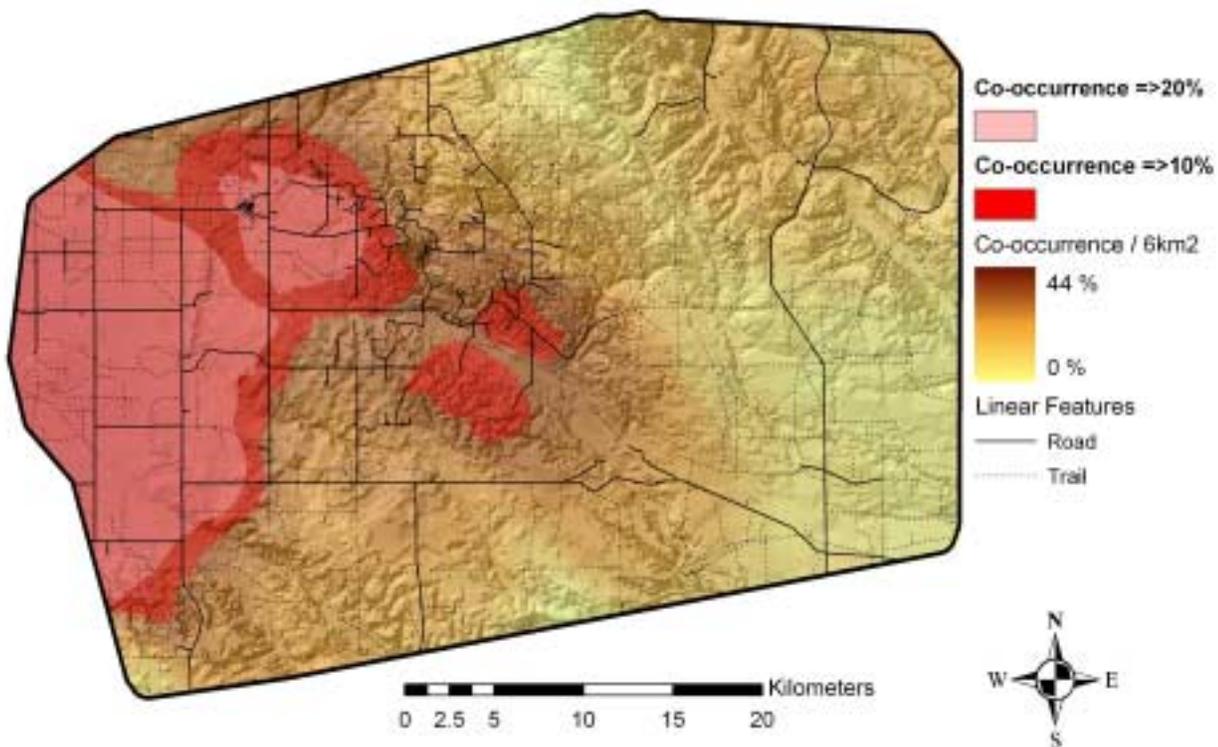


Figure 6. Pixels predicted to contain the co-occurrence of both sage-grouse and mosquitoes shown for the top co-occurrences (yellow to brown gradient) covering at least 10% of the 6 km² area (red), and covering at least 20% of the area (pink). The proportion of co-occurrence for sage-grouse and mosquitoes per 6 km² is shown for reference.

This resulted in many different potential options for the placement of our two mosquito traps. However, in an effort to make the sites more specific to 2004, and thus increase the probability of female sage-grouse occurring in our sample areas, we chose two sites that currently each have five different females on nests and incubating eggs within the sample areas and multiple other nests surrounding each area (Figure 7, sample areas A and B).

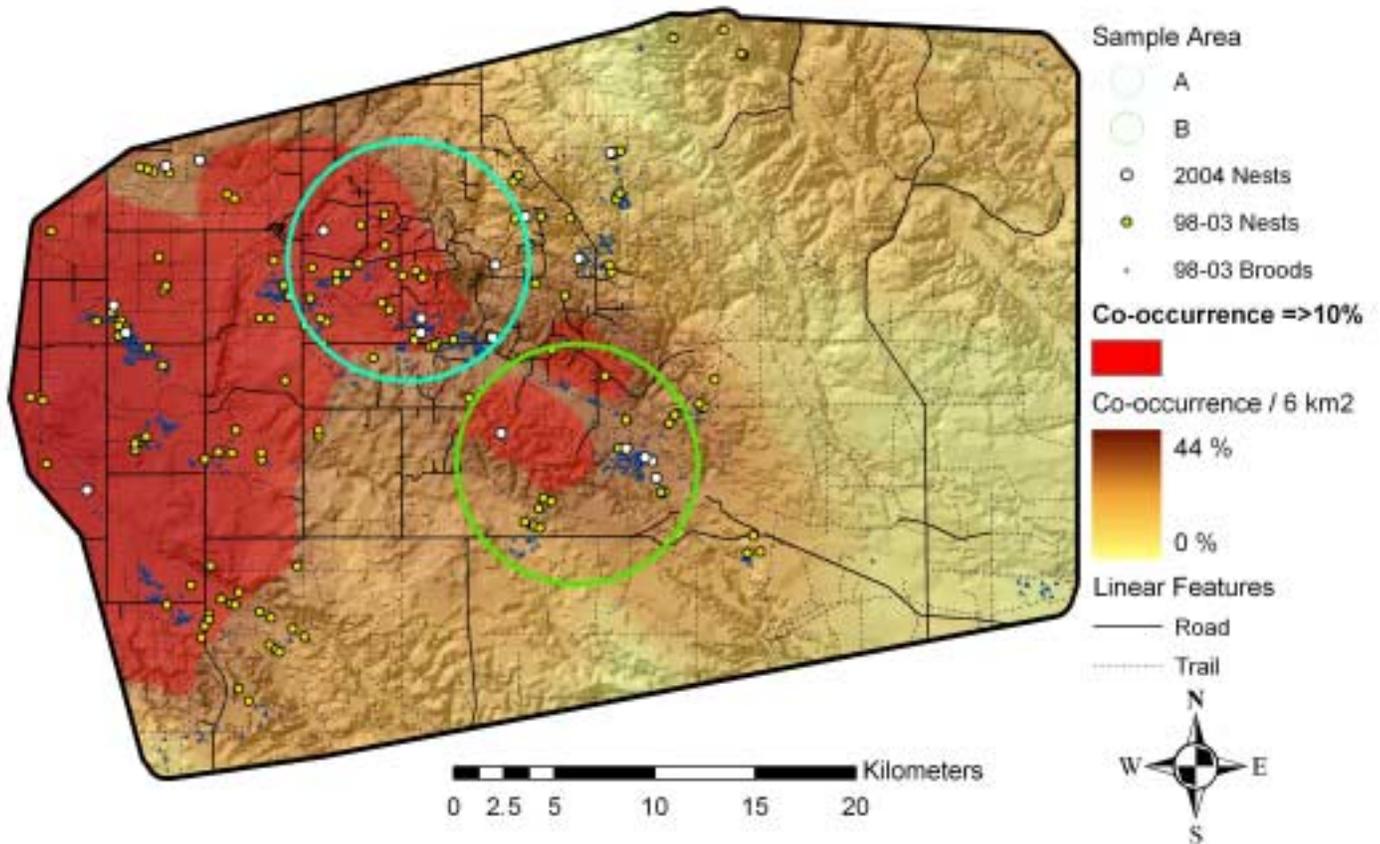


Figure 7. Proposed sample areas (large circles) overlaid upon the co-occurrence of both sage-grouse and mosquitoes. Previous nest and brood locations (1998-2003) are shown to for reference to habitat use, and current (2004) nesting sage-grouse (2004 failed nests not displayed) are shown (white circles) to illustrate females with potential broods in 2004. The proportion of co-occurrence (per 6 km²) for sage-grouse and mosquitoes (yellow to brown gradation) and top co-occurrences covering at least 10% of the 6 km² area (red) are shown for reference.

The centroids of the sample areas A and B are about 14 km apart, leaving a *ca.* 2 km buffer between the two areas. Both sites are similar in co-occurrence values (sage-grouse use and wet CTI index; A = 12.4%, B = 10.1%). Looking at sage-grouse use alone, both sites have a high proportion of the area comprised of RSF scores ≥ 9 (A = 29.6%, B = 22.6%), and about both have a similar proportion of the sample area with wet CTI index values (CTI ≥ 10 ; A = 24.2%, B = 28.3%). Similarly, both sample areas contained a comparable amount of water (A = 2.99%, B = 3.54%).

Field Methodology

Two CDC mosquito surveillance traps will be placed that the center of each 6 km² sample area, with one placed 500 m east of the sample centroid and one placed 500 m to the west (1 km apart). Traps will be activated and samples collected for one 12 hour overnight period of each week (preferable Tuesday). Trapping will begin on 01-June-2004, lasting up to 18 weeks, depending on mosquito emergence. Traps will be emptied the following morning, and samples shipped into the Medicine Alberta Environment office, before being sent to the Provincial Lab in Calgary for mosquito identification and WNV testing.

One of the two sample areas will be randomly chosen and treated with Bti (*Bacillus thuringiensis israelensis*) larvicide granules to control mosquito populations, and thus, the risk of sage-grouse becoming infected with WNV. Bti will be applied to all shallow standing water within the 6 km² treated sample area, with an application rate of about 3 kg/ha, or $< 1\text{g/m}^2$. We estimate that it will take approximately one week to apply the Bti to the entire treatment sample area, but this will depend on precipitation. We will

apply Bti to the entire area on three separate occasions over the mosquito season, reducing mosquito abundance throughout the summer. The first treatment will coincide with the initial mid-June peak mosquito hatch, and the subsequent treatments will occur two and four weeks later, in early and mid-July, respectively.

Application

We feel that this approach offers us the best opportunity to 1) identify two sample areas with similar characteristics (biogeographic, water availability, and probability of sage-grouse use during the summer), 2) identify two sample areas with similar mosquito abundances (prior to any treatment), 3) apply larviciding treatment across either one of these sites with a high probability of reducing mosquitoes, and thus, the threat of WNV to the endangered sage-grouse, 4) obtain results that have the greatest likelihood of identifying management benefits for sage-grouse in the face of the newly discovered threat of WNV.

Budget

The total amount of water in each 6 km² sample area (A = 2.99%, B = 3.54%) was about 400 ha. However, most of these larger water bodies will not be suitable mosquito habitat (i.e water too deep, water too exposed with wave action, etc.). Also, most of the creeks are intermittent, and thus, only small remaining pools of standing water left behind will remain as suitable mosquito habitat. Thus, we estimate that 10-20% of the water bodies we have identified will 1) still have water in them, and 2) be suitable mosquito habitat. Thus, we estimate we will need to treat between 40 and 80 ha

of water. With Bti costing \$145/20 kg bag (Aquabac 200G Biological Larvicide Granules (PCP# 26863) - TrueNorth Specialty Products), and an application rate of about 3 kg/ha, we estimate the cost of Bti for a single application will be between \$870-\$1,740. Thus, we for three applications, estimate the total cost of Bti will be between \$2,610-\$5,220.

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