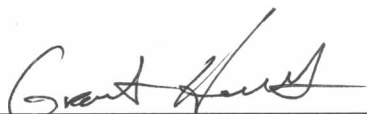


Risk mapping of bluetongue virus in
Montana using habitat suitability for
Culicoides vectors

Pete Edminster
Carroll College
Dept. of Natural Sciences
Honors Thesis
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This thesis for honors recognition has been approved for the
Department of Natural Sciences by:



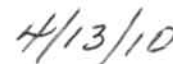
Dr. Grant Hokit, Director,
Department of Natural Sciences



Date



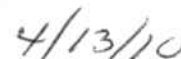
Dr. Gerald Shields, Professor,
Department of Natural Sciences



Date



Mr. Jack Oberweiser, Professor,
Department of Mathematics



Date

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Abstract

Bluetongue disease, first reported in the United States in 1952, affects cattle, sheep, and other wild and domesticated ruminants. Globally, the biting midge (order *Diptera*, family *Ceratopogonidae*, genus *Culicoides*) has been identified as the primary vector for the spread of bluetongue. Preliminary research has demonstrated that outbreaks of arboviruses can be accurately predicted using habitat characteristics essential to vector survival, as global disease occurrence is closely correlated to distribution of the disease vector. The goal of my study was to derive an accurate map of statewide habitat suitability for *C. sonorensis* based on individual environmental factors, which could then be used to infer bluetongue risk. I used the program MaxEnt to build a statewide habitat suitability model for the biting midge, *Culicoides sonorensis* using environmental data layers and presence-only data for known locations throughout Montana. Modeling with MaxEnt resulted in a statewide map of *C. sonorensis* habitat with an AUC=0.928, demonstrating a strong correlation between the model and the presence data. Environmental variables incorporated into the final model included distance to surface water, land cover, slope, and elevation. The MaxEnt output of midge habitat suitability shows highly suitable habitat in eastern Montana, which becomes drastically less hospitable in the western part of the state, presumably due to higher elevation, which is known to limit midge distributions. Although this project produced a highly significant model, the low amount of presence data for *C. sonorensis* resulted in a limited number of training points for modeling analysis, and should be expanded in order to further legitimize the results and allow for use of test data.

Introduction

Bluetongue viral disease, first reported in the United States in 1952, affects cattle, sheep, and other wild and domesticated ruminants (Walton and Osburn, 1992; Gibbs & Greiner, 1994). Originally called “soremuzzle”, bluetongue was first described in South Africa in the early 1900’s, but did not occur outside Africa until 1942, when an outbreak was reported in Cyprus. Since then, significant bluetongue outbreaks have occurred throughout Europe, the Middle East, Asia, and North America (Sabirovic et al, 2008; Mellor, 1990). In Montana, the most recent bluetongue outbreak arose in 2007 in Musselshell County and spread to 15 other counties (Figure 1) where domestic sheep herds were hardest hit, along with reported cases in deer and pronghorn antelope (Zaluski, 2007). Symptoms may vary among serotypes, but generally include fever, open sores or hemorrhages on the tongue, mouth, and nostrils, swollen red-blue coronary bands around the horns and feet accompanied by lameness, muscular atrophy, loss of wool, and death (Zaluski, 2007; APHIS, 2003). Swelling and hemorrhaging of the mucous membranes can give the mouth a characteristic blue color, leading to the name bluetongue disease.

Bluetongue can inflict major damage to livestock-based economies through loss of animals and implementation of trade restrictions. Livestock producers in the US alone lose an estimated \$125 million each year due to limitations on trade and costs for animal testing (Stelljes, 1999). However, proper prevention and preparation can greatly reduce the risks and impacts of bluetongue on a local scale. Multiple vaccines were developed for use in controlling bluetongue outbreaks in Europe with significant success, but application is limited since vaccines are generally specific to a certain serotype (Walton

and Osburn, 1992; Savini et al, 2008). Also, land management strategies can be used to eliminate vector breeding and resting sites, as well as use of insecticides and protective housing strategies for livestock (Carpenter et al, 2008).

Globally, the biting midge (order *Diptera*, family *Ceratopogonidae*, genus *Culicoides*) has been identified as the primary vector for the spread of bluetongue (Foster et al, 1963). Many but not all species within *Culicoides* have the potential to transmit the virus including *C. sonorensis* (previously *C. variipennis sonorensis*), which is most prevalent in the western United States (Mellor, 1990). While extensive research into the life cycle of midges has been done, much is still unknown about midge behavior in the adult stage. Adult females are crepuscular feeders and seek out blood meals, while males feed on nectar (Mellor et al, 2000). After ingestion of a blood meal necessary for oogenesis, females develop and deposit eggs in moist environments ranging from standing water to rotting fruit (Mellor et al, 2000). Larvae generally emerge 2-3 weeks after warm temperatures arrive in the summer, and the adult stage follows within a week (Lysyk, 2007). Adults can live up to 90 days after hatching, and the females reproduce multiple times within this lifespan (Mellor et al, 2000).

The bluetongue virus has a double protein coat; the outer coat aids in endocytosis into target host cells, delivering the inner core capsid to the cytoplasm (Forzan et al, 2007). Little is known of the specific mechanism of bluetongue cell entry, its replication, or its life cycle after infection of a ruminant host. The bluetongue cycle within the midge occurs as follows: infection results from ingesting a blood meal from an infected host, the virus replicates in the midgut tissue of the midge, secondary infection of salivary glands and other tissues follows, and within 10-14 days viral titer is sufficient for transmission

of the virus to a new host, which is achieved at a subsequent blood meal via saliva transfer (Mellor, 1990). Thus, development of the bluetongue virus in *Culicoides* requires consumption of a blood meal, survival of the virus incubation cycle, and oviposition followed by a second blood meal, which transmits the mature virus to the next host (Gerry and Mullens, 2000). Adult insect survival through this cycle is largely dependent on ambient temperatures, and abundance is often positively correlated with temperature, resulting in a seasonal transmission pattern of bluetongue (Osburn et al, 1981; Lysyk, 2007). Also, since bluetongue is not inherently fatal in midges, the individual can remain infected and actively transmit the virus for the remainder of its life (Mellor, 1990). However, oral infection does not guarantee vectorial capacity, since research has shown that infected individuals can still fail to transmit the virus, and subsequent studies are underway to determine the mechanisms for this discontinuity (Gerry et al, 2001; Mellor et al, 2000).

Spatial epidemiology is the analysis of variation in disease risk and incidence relative to environmental, behavioral, and demographic factors (Elliott and Wartenberg, 2004; Ostfeld et al, 2005; Nature Serve, 2007). Preliminary research has demonstrated that outbreaks of arboviruses can be accurately predicted using habitat characteristics essential to vector survival, as global disease occurrence is closely correlated to distribution of the disease vector (Mellor, 1990). Tachiiri et al (2006) used environmental factors along with abundance of vector and reservoir species to predict risk of West Nile Virus in British Columbia, Canada. The same technique has been used to analyze risk of bluetongue in incursive zones including Sicily (Purse et al, 2004) and the northern United States (Green et al, 2005). Analysis of environmental factors can thus shed light on the

transmission of bluetongue virus and its possible spread into areas that have previously had no significant occurrence. Habitat mapping of populations of *C. sonorensis* based on environmental suitability would allow health officials to analyze localized risk, and may contribute to risk maps that identify high-risk areas within Montana. With this knowledge, veterinarians and ranchers can better prepare for bluetongue and its gradual spread.

The goal of my study was to derive an accurate map of statewide habitat suitability for *C. sonorensis* based on individual environmental factors. In doing so, I hypothesized that if environmental conditions favorable for survival and growth of *C. sonorensis* can be quantified at known *C. sonorensis* locations, then these conditions can be used to predict *C. sonorensis* presence elsewhere.

Materials and Methods

I used the program MaxEnt (Phillips and Dudik, 2008) to build a statewide habitat suitability model for the biting midge, *Culicoides sonorensis*, using environmental data layers and presence-only data for known locations throughout Montana.

Sample Collection: Coordinates for *C. sonorensis* locations were obtained from Johnson (2004) in which samples were collected at locations across Montana using miniature, battery operated black light traps and 1 kg of dry ice. Traps were placed in close proximity to larval habitats, and operated from dusk to dawn on two consecutive nights. Resulting samples were then sorted to determine the total number of *C. sonorensis* present. Locations at which no midges were collected were excluded from this study, resulting in 28 total data points. These resulting sites which were positive for *C. sonorensis* were entered into a spreadsheet file using latitude and longitude coordinates

provided by Johnson (2004). The spreadsheet was converted to CSV (comma delimited) format, which was then used to create point locations using a geographic information system (GIS).

Environmental Variables: Environmental layers were obtained from various sources (a summary of source information and relevant metadata for all environmental layers is provided in Appendix A) and adapted to ASCII format using GIS software (Arcview 9.3). All environmental layers were resampled to 90m x 90m square plots for the entire state of Montana. This input format is required for use in MaxEnt (Phillips, 2004). A pilot area was used in preliminary analysis of layer suitability, statistical significance, and overall program function. Environmental layers were chosen based upon both performance in the pilot model and known significance to midge habitat. Some variables were later eliminated due to over-fitting the model. The final model utilized both continuous and categorical variables including distance to surface water (Gerry and Mullens, 2000; Gerry et al, 2001) maximum and minimum temperature and soil composition (Lysyk, 2006; Lysyk, 2007), humidity (Wittman et al, 2002), elevation (Green et al, 2005), slope (Mullens, 1989), precipitation, wind power, wind speed and land cover.

Model Development: MaxEnt uses maximum entropy theory, presence-only data, and environmental data to determine habitat suitability and project probability that a particular species will occur at any given point (Phillips et al, 2004). In this way, MaxEnt has been shown to model species distributions very accurately, and performs better than other modeling techniques (Kumar et al, 2008; Maxell, 2004). Sample locations were included using latitude and longitude coordinates. Each environmental layer provided

data for a specific variable for every 90m x 90m quadrant throughout the state. MaxEnt allows input of multiple variables, which are then evaluated cumulatively according to Gibb's Distribution (Phillips et al, 2004). MaxEnt then provides the option of various visual output formats, including raw, logistic, and cumulative data. A logistic scale output was used for this study because it can easily be interpreted as the probability of occurrence at a given point. MaxEnt allows the option of using some samples to "train" the model and then a smaller percentage of the samples can be used to test the model (random test percentage). However, due to the limited sample size (28 locations) I did not designate a test percentage and all sample locations were used to train the model.

Model Evaluation: The MaxEnt output provides a series of tables and figures illustrating overall fit and statistical relevance of the model. Both the collective model and each individual variable were analyzed for correlation with the known presence data points. The "jackknife" output (Figure 2) analyzed the quality of the model excluding only a particular variable, as well as with that variable alone (Phillips et al, 2004). This was used to determine the relevance of individual variables to the model, ultimately leading to inclusion or exclusion of the particular variable. The AUC (area under curve for sensitivity vs. specificity) expresses that probability that a given point will have a higher probability of occurrence than random chance (Maxell, 2004). AUC was used to quantify the overall fit of the model to the training data (Phillips et al, 2004), and was used as the final gauge of model significance (Figure 3). An AUC value above 0.5 indicates that the model predicts midge location better than random chance, where a value of 1.0 would indicate perfect predictability.

Results

Modeling with MaxEnt resulted in a statewide map of *C. sonorensis* habitat (Figure 4) with an AUC=0.928, demonstrating a strong correlation between the model and the presence data. No test AUC was generated.

Environmental variables incorporated into the final model included distance to surface water, land cover, slope, and elevation (Figure 5). Midge abundance had a strong inverse correlation with distance to surface water, and a significant probability of presence (hereafter considered above 0.5) was observed only for distances below 350m. A peak probability of 0.774 was observed at a distance of 0 m. Five distinct classes of land cover showed significant suitability for midge habitat: low-intensity developed vegetation (0.835), intermountain basin/greasewood flats (0.811), northwestern Great Plains riparian (0.809), introduced upland vegetation/forblands (0.630) and northwestern Great Plains mixed grass prairies (0.534). Three other vegetation types, cultivated cropland (0.443), northern rocky mountain lower montane/foothill and valley grasslands (0.249) and inter-mountain basins/big sagebrush steppes (0.220), showed elevated but non-significant probabilities. MaxEnt outputs revealed a limited range of slope ideal for midge survival. Significant probability of midge presence was observed for slopes between 0.864 and 3.056, and peak probability of 0.571 at a slope of 1.063. Although its effects were clearly evident on the risk map, elevation had the least explanatory power of the environmental variables included in the model, and demonstrated an inverse relationship to probability of midge presence. Probability was significant at elevations below 1000m.

Precipitation, humidity, wind power, wind speed and temperature (both max and min) variables were excluded due to poor early performance in the model. The soil

compositions layer, while significantly correlated with midge occurrence, was excluded due to incomplete data that resulted in blackouts of predicted habitat suitability for certain areas. While this layer was consistently one of the most significant, its removal did not greatly affect the overall fit of the model.

Discussion

The MaxEnt output of midge habitat suitability shows highly suitable habitat in eastern Montana, which becomes drastically less hospitable in the western part of the state, presumably due to higher elevation, which is known to limit midge distributions (Green et al, 2005). This result correlates strongly with the 2007 Montana bluetongue outbreak, which started in Musselshell County and spread almost exclusively eastward to the areas which are designated suitable midge habitat based on this study (Figure 1; Zaluski, 2007).

All environmental variables included in the final model had previously-known associations with midge habitat with the exception of land cover.

Surface Water: Midges were limited most significantly by distance to surface water due to their dependence on standing water for oviposition and larval development (Mellor et al, 2000). Thus, elimination of standing pools and breeding sites as a means of controlling the spread of *Culicoides* on a local scale has been suggested as an effective means of combating the spread of bluetongue (Carpenter et al, 2008). However, species of *Culicoides* are known to breed in any relatively moist environment, including dung, making elimination of breeding sites in agricultural areas nearly impossible, and suggesting that another relationship with surface water may exist that further limits distribution (Mellor et al, 2000; Carpenter et al, 2008).

Land cover: While the effects of different types of vegetative land cover in relation to *Culicoides* distribution has not been studied, it played a large role in the model distribution. Differing vegetation types could be associated with particular soil compositions favorable to midge survival (Green et al, 2005) or host abundance. The results demonstrate an overlap between vegetation ideal for both midge habitat and that of host livestock. Also, vegetation could play a role in preventing wind dispersal, which is known to affect midge populations and distributions (Walton and Osburn, 1992; Mellor et al, 2000).

Slope: The slope of surrounding landscape can have a variety of effects on midge habitat. First, a gentle-sloping or level surface allows for collection of standing water, the importance of which in the life cycle of the midge is discussed above. Second, Lysyk (2006) and Mullens (1989) showed that shallow-sided pools are needed for successful oviposition and larval development to occur, and accumulation of such pools may be encouraged in gently sloping terrain.

Elevation: Green et al (2005) demonstrated that herds within given altitudes in the neighboring states of North Dakota, South Dakota, and Nebraska had significantly higher seropositivity levels for bluetongue, and that this relationship existed within a limited range of ideal altitudes. Green et al (2005) suggested that this relationship might have more to do with other environmental factors, such as humidity and temperature, which are related to elevation and play a significant role in midge survivorship (Wittmann et al, 2002). While elevation contributed the least to the final model, excluding this variable had significant detrimental effects on the model performance. Also, the MaxEnt visual output showed a drastic decrease in midge habitat in western Montana where elevations

rise steeply (USGS), further suggesting the importance of this variable as a limiting factor in midge distribution.

Many environmental variables that I expected to play a large role in my risk map were eliminated from the model due to failure to contribute to the predicted distribution, or a detrimental effect on model performance. The known effects of some of these variables on midge survival demand addressing this contradiction. One common confounding property in maximum entropy modeling involves interference between closely related environmental variables, causing over fitting (Phillips et al, 2004). Some variables, such as humidity, precipitation, wind power and wind speed are presumed to have interfered or interacted with other more significant variables, negating their contribution to the final model. Humidity and precipitation may have been significantly related to elevation so that their inclusion in the model caused redundancy and decreased model performance. Also, wind power and wind speed, which were expected to have some effect on suitable midge habitat, may have been negated by the presence or absence of particular classes of land cover which would function as wind breaks. Eliminating these redundant layers decreased the likelihood of such errors.

Temperature: The vital importance of temperature on midge survival and reproduction, not to mention the viral replication cycle of bluetongue, is well documented, and I expected this variable to play the largest role in predicting suitable midge habitat (Gerry et al, 2000; Wittmann et al, 2002). Lysyk (2007) observed that *C. sonorensis* emerged only after ambient temperatures rose above 0°C for two weeks, and population peaks were first observed when temperatures first rose above 16°C. The layers used in our analysis documented the average maximum and minimum July temperatures from 1971-

2000, which may have neglected the important factors uncovered by Lysyk, and thus failed to contribute significantly to midge habitat in our model. Development and application of a more appropriate layer for temperature could further enhance the performance of the model.

Soil Composition: Though not fully understood or thoroughly tested, some links have been made between soil compositions and conditions favorable to larval midge development. Lysyk (2006) identified an increased incidence of *C. sonorensis* in soils of high salinity, and while MaxEnt identified 25 soil categories that showed elevated probability of presence (above 0.5), this association was likely due to overfitting. During preliminary modeling this layer was consistently the most significant contributor to the model, but often caused over fitting and blackouts of large portions of Montana due to incomplete sampling and data. The soils layer was eventually eliminated, and the small resulting decrease in model performance was deemed acceptable.

Although this project produced a highly significant model (AUC=0.928), the low amount of presence data for *C. sonorensis* resulted in a limited number of training points for modeling analysis, and should be expanded in order to further analyze the results. Along with minimizing error, further sampling would allow for use of test data to check the accuracy of the resulting model. In modeling with less than 100-125 sample points, all the data must be used to train the model, and specifying test points compromises training. Designating test data allows MaxEnt to use a certain percentage of sample points to test the validity of the model by comparing these leftover presence locations with the predicted habitat distributions. The resulting test AUC is a more accurate measure of model performance than training AUC (Phillips et al, 2004).

My model predicts suitable midge habitat, which can be used to further infer risk of bluetongue incidence. Due to other factors that may affect bluetongue risk in a given area (e.g. the presence of livestock or wild ruminants), this model may overestimate risk throughout the state. A follow-up analysis using training data that correspond to points of bluetongue incidence would provide a more robust model of the degree of infection risk. Such an analysis should incorporate layers that reflect factors that affect bluetongue transmission, such as vector habitat (a layer that was produced here), temperature, livestock density, land use, etc. Up to this point such an analysis is not possible due to the low number of independent bluetongue outbreaks that have occurred in the state. Regardless, my model can serve as a starting point for inferring the most likely locations for bluetongue disease in Montana.

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Appendix A: Description of Environmental Data Layers

Environmental data layers for this study included nine continuous (distance to surface water, maximum temperature, minimum temperature, humidity, elevation, slope, precipitation, wind power and wind speed) and two categorical (soil composition, land cover) variables. These layers were converted to raster format with 90m grid cells using GIS software, which resulted in 10,204 columns and 892 rows. All layers were then converted to ASCII grid format to be used in MaxEnt. Source information and links, as well as relevant metadata are provided below. True metadata are available by download from the source links provided.

Distance to Surface Water: This data layer was derived from two hydrography datasets. The first, a high-resolution layer, was used primarily, while the second, low resolution layer was used when no high-resolution data were available. A function in GIS was then used to create a rasterized layer that incorporated minimum distance to surface water. Hydrography datasets were created by the US Geological Survey and US Environmental Protection Agency, and are available from National Resource Information System (<http://nris.mt.gov/gis/gisdatalib/gisDataList.aspx>).

Maximum Temperature: This dataset described estimated average daily maximum temperature in degrees Fahrenheit during the month of July from 1971-2000 statewide. Estimates were derived based on known point data combined with Parameter-elevation Regressions on Independent Slopes Model (PRISM), which uses a digital elevation model (DEM), to project local climatic conditions. The layer was constructed by the Oregon Climate Service at Oregon State University, and original data can be downloaded

from <http://www.ocs.orst.edu/prism>. The data used in this study was resampled to 600m resolution and is available at <http://nris.mt.gov/gis/gisdatalib/gisDataList.aspx>.

Minimum Temperature: This dataset described estimated average daily minimum temperature in degrees Fahrenheit during the month of July from 1971-2000 statewide. Estimates were derived based on known point data combined with Parameter-elevation Regressions on Independent Slopes Model (PRISM), which uses a digital elevation model (DEM), to project local climatic conditions. The layer was constructed by the Oregon Climate Service at Oregon State University, and original data can be downloaded from <http://www.ocs.orst.edu/prism>. The data used in this study was resampled to 600m resolution and is available at <http://nris.mt.gov/gis/gisdatalib/gisDataList.aspx>.

STATSGO Soil Composition: The STATSGO layer was a general map of soil coverage derived from more specific coverage maps, geological and ecological characteristics, and probable classification based on Land Remote Sensing Satellite (LANDSAT) images. Final soil composition was determined by sampling or transecting areas and statistically projecting soil coverage. Minimum delineated area was 625 ha. The layer was created as a part of the National Cooperative Soil Survey by the U.S. Department of Agriculture in 2006. Further data are available from <http://nris.mt.gov/gis/gisdatalib/gisDataList.aspx>.

Humidity: Data for this layer were available for the contiguous United States from the Numerical Terradynamic Simulations Group (University of Montana) website (<http://www.daymet.org>). Data were then clipped to include only Montana, and modified in GIS to fit MaxEnt specifications.

Elevation: This layer is a 30m raster grid created by the US Geological Society, though the source data had to be resampled to 90m for this study. The original layer is available from NRIS (<http://nris.mt.gov/gis/gisdataLib/gisDataList.aspx>).

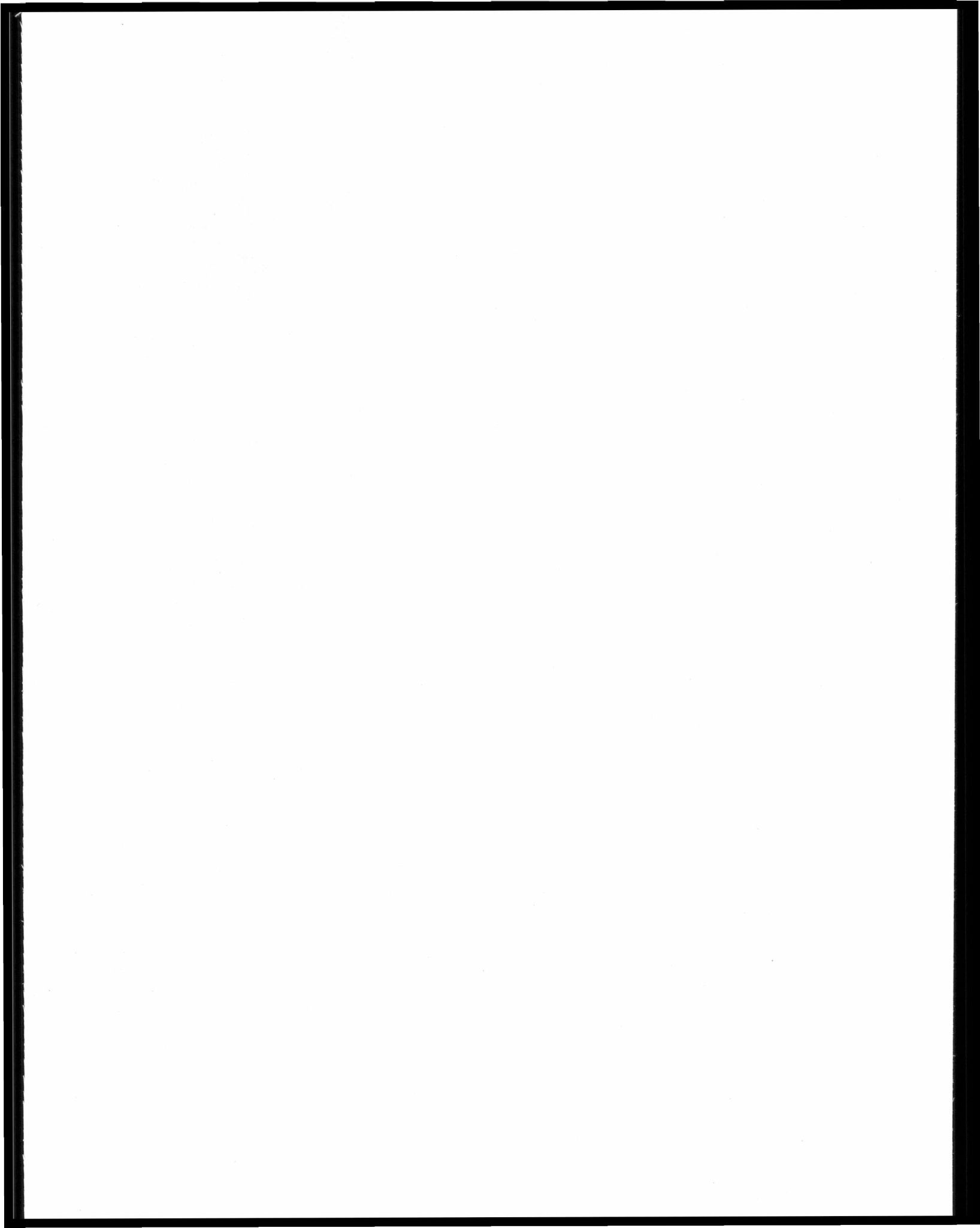
Slope: The slope layer, measured in degrees from the horizontal, was derived from the elevations layer using the slope function in GIS. A description of the elevation layer used is included above.

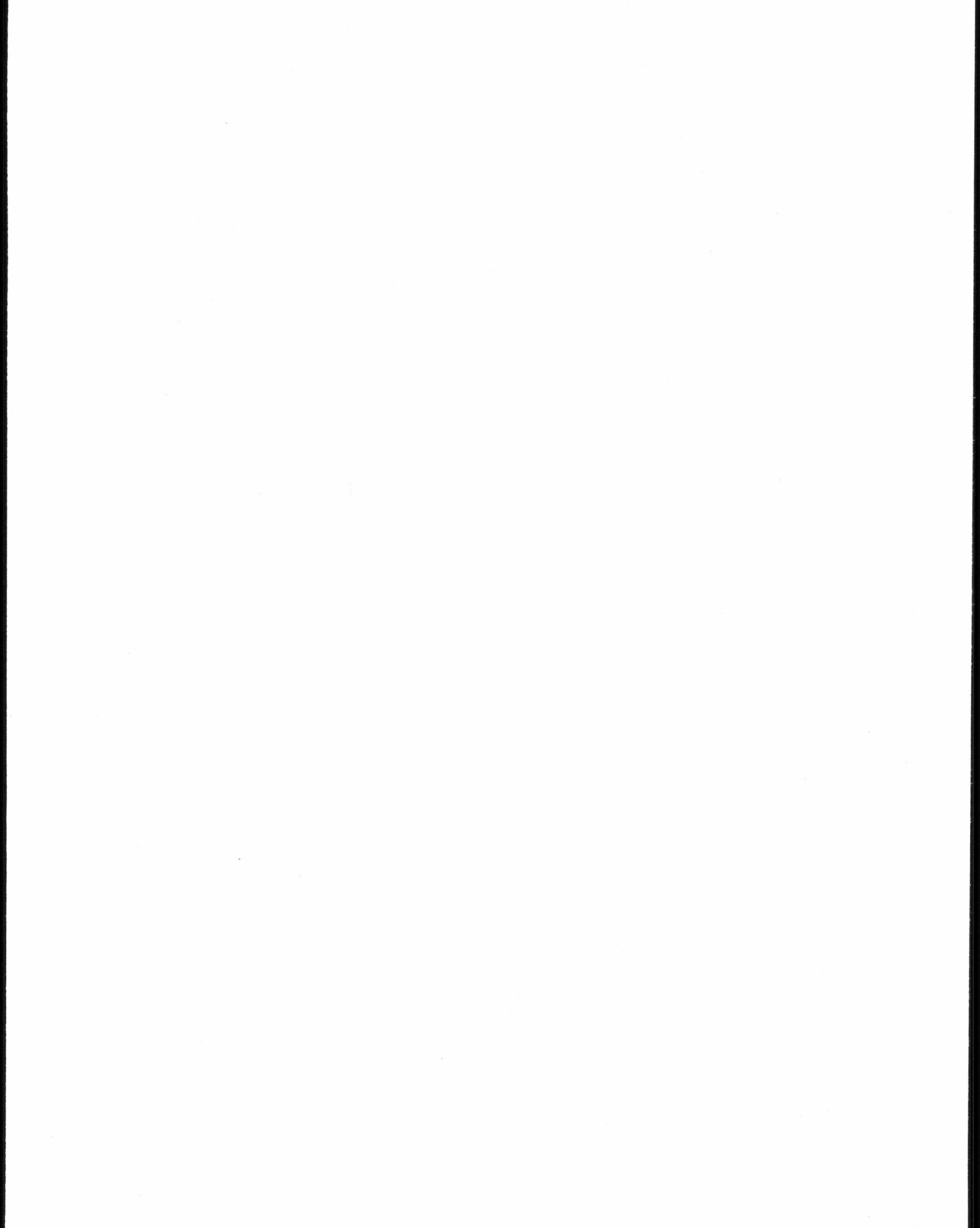
Relative Effective Annual Precipitation: This layer estimates relative precipitation based on average soil moisture at 1cm intervals between 1971 and 2000. Estimates are based on data from the University of Montana's Numerical Terradynamics Simulation Group (NTSG) available from DAYMET (<http://www.daymet.org>). Precipitation data from 1980 to 1997 were adjusted to the period of 1971-2000 using the National Water and Climate Center's Temperature and Precipitation Summary Tables (TAPS), and sensitized based on local characteristics such as slope, aspect, and soil composition. Precipitation estimates were developed for each county in Montana and pieced together by the Montana State Office of Natural Resources Conservation Service. The layer is available from NRIS (<http://nris.mt.gov/gis/gisdataLib/gisDataList.aspx>).

Wind Power: TrueWind Solutions created this layer in 2002. It measures average wind power in watts/m² at 50m above the ground at a pixel size of 400m, resulting in a raster grid with 1338 rows and 2308 columns. However, the pixel size was adjusted to 90m for use in this study. Data were collected using the Mesomap system, as well as historical records. The original data layer is available from NRIS (<http://nris.mt.gov/gis/gisdataLib/gisDataList.aspx>).

Wind Speed: TrueWind Solutions created this layer in 2002. It measures average wind speed in m/s at 50m above the ground at a pixel size of 400m, resulting in a raster grid with 1338 rows and 2308 columns. However, the pixel size was adjusted to 90m for use in this study. Data were collected using the Mesomap system, as well as historical records. The original data layer is available from NRIS (<http://nris.mt.gov/gis/gisdata/lib/gisDataList.aspx>).

Land Cover: Land cover was assessed in 90m grid squares with a 10km allowance at the border for adequate edge-matching with neighboring states. The Wildlife Spatial Analysis Lab at the University of Montana used aerial photography to categorize and analyze land cover characteristics. Two subsequent analyses were used to categorize land cover: an unsupervised analysis to establish patch boundaries followed by a supervised analysis to assign land cover labels. Slope, aspect, and elevation were considered during analysis, as well as confounding factors such as cloud cover and presence of urban areas. The original layer is available from NRIS (<http://nris.mt.gov/gis/gisdata/lib/gisDataList.aspx>).





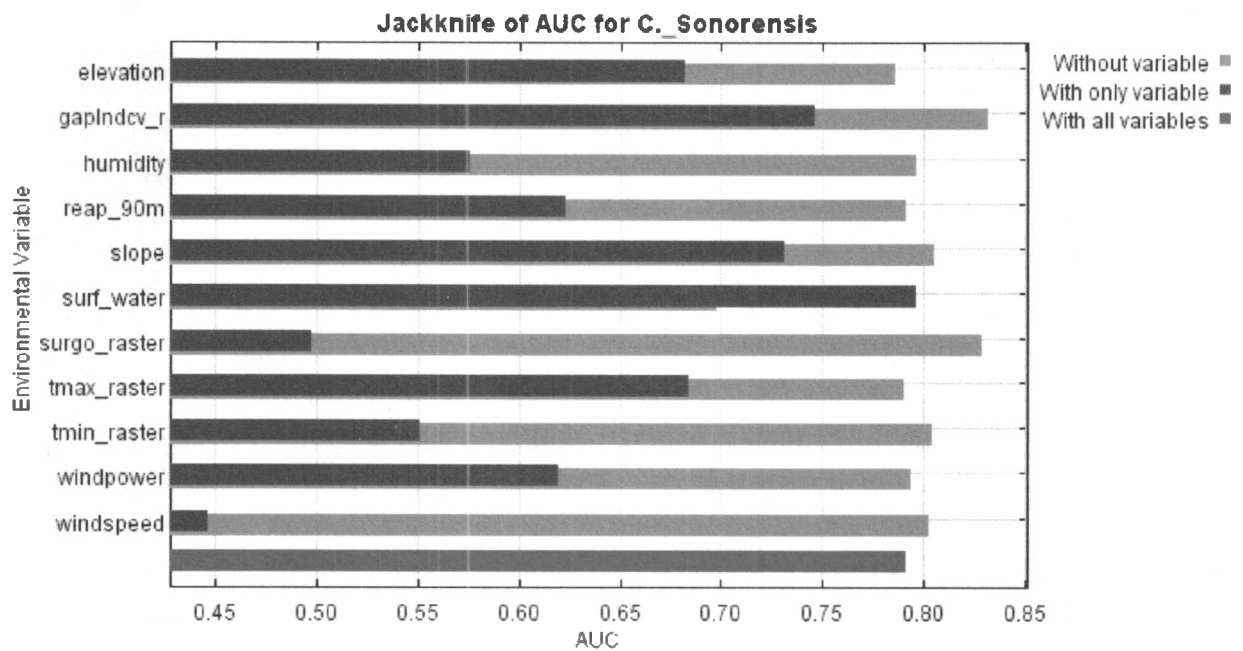
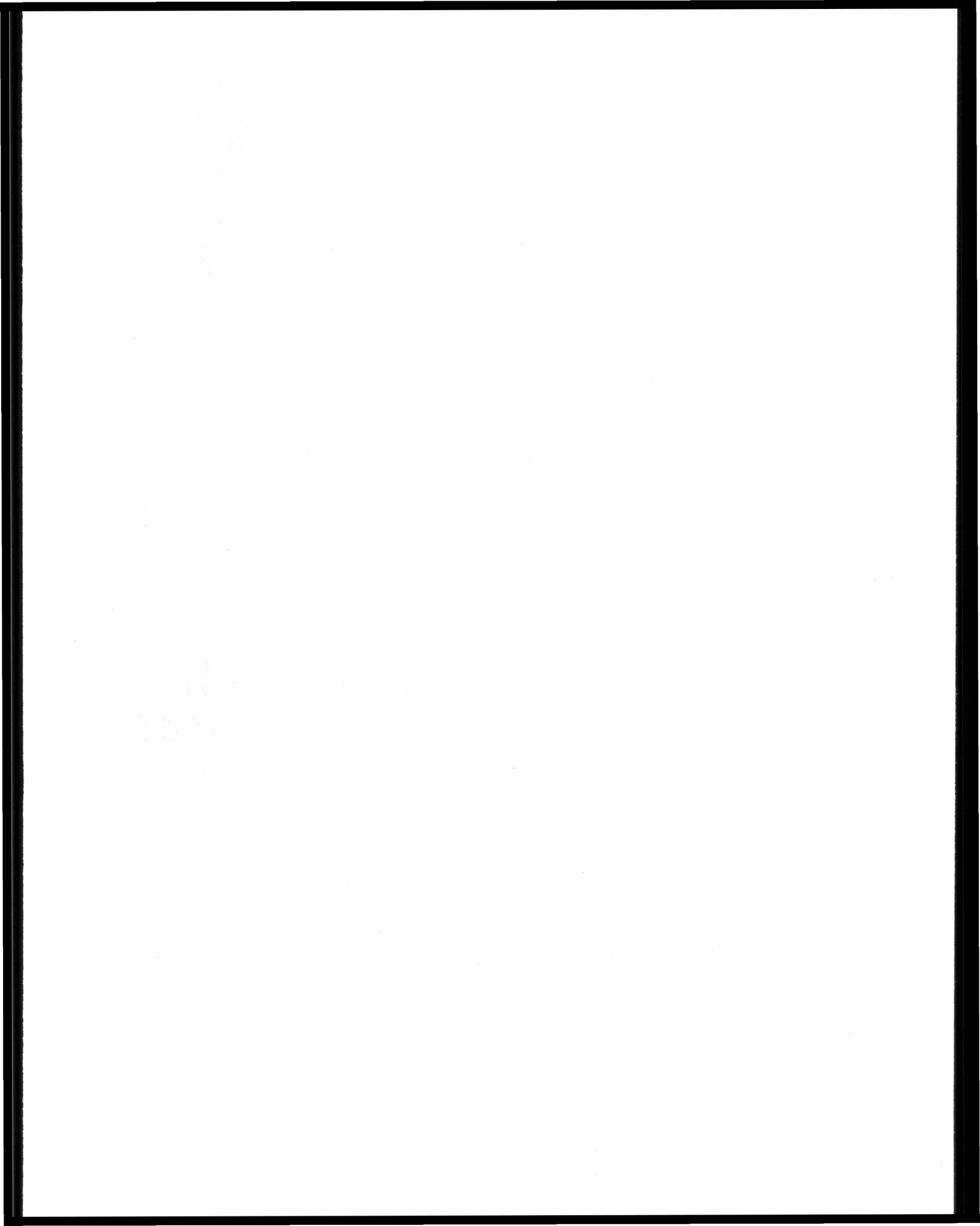


Figure 2: A preliminary jackknife output evaluating individual layer performance based on AUC (y-axis) relative to overall model (red). Models with strong correlations show either small differences between blue and red bars, or larger blue bars than green.



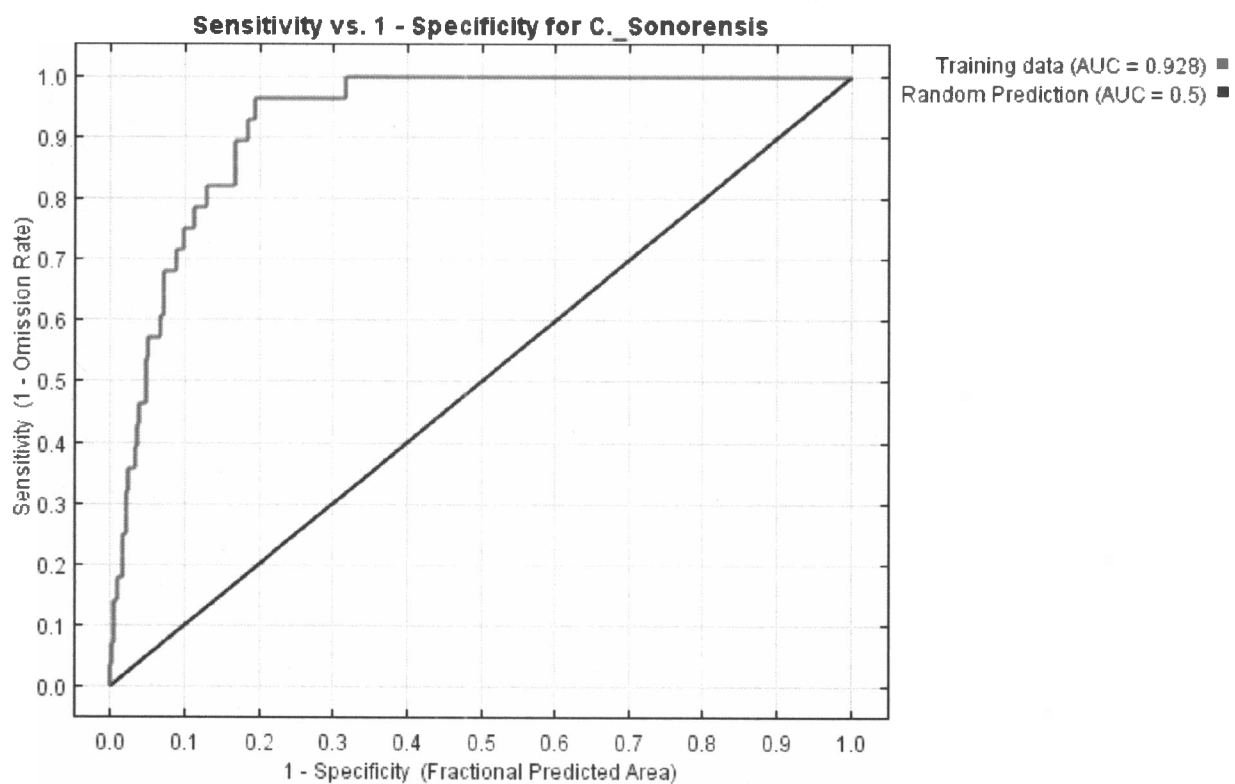
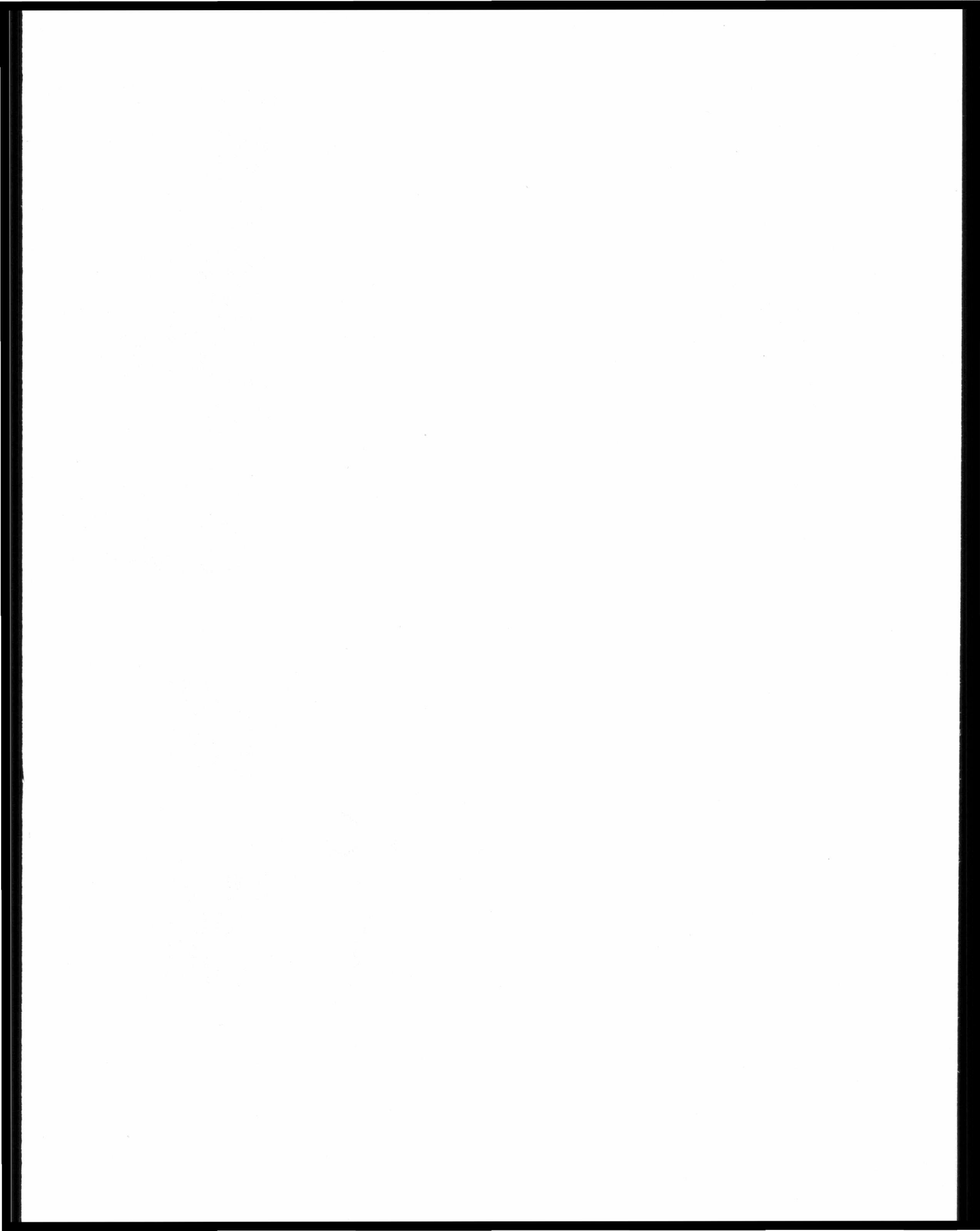
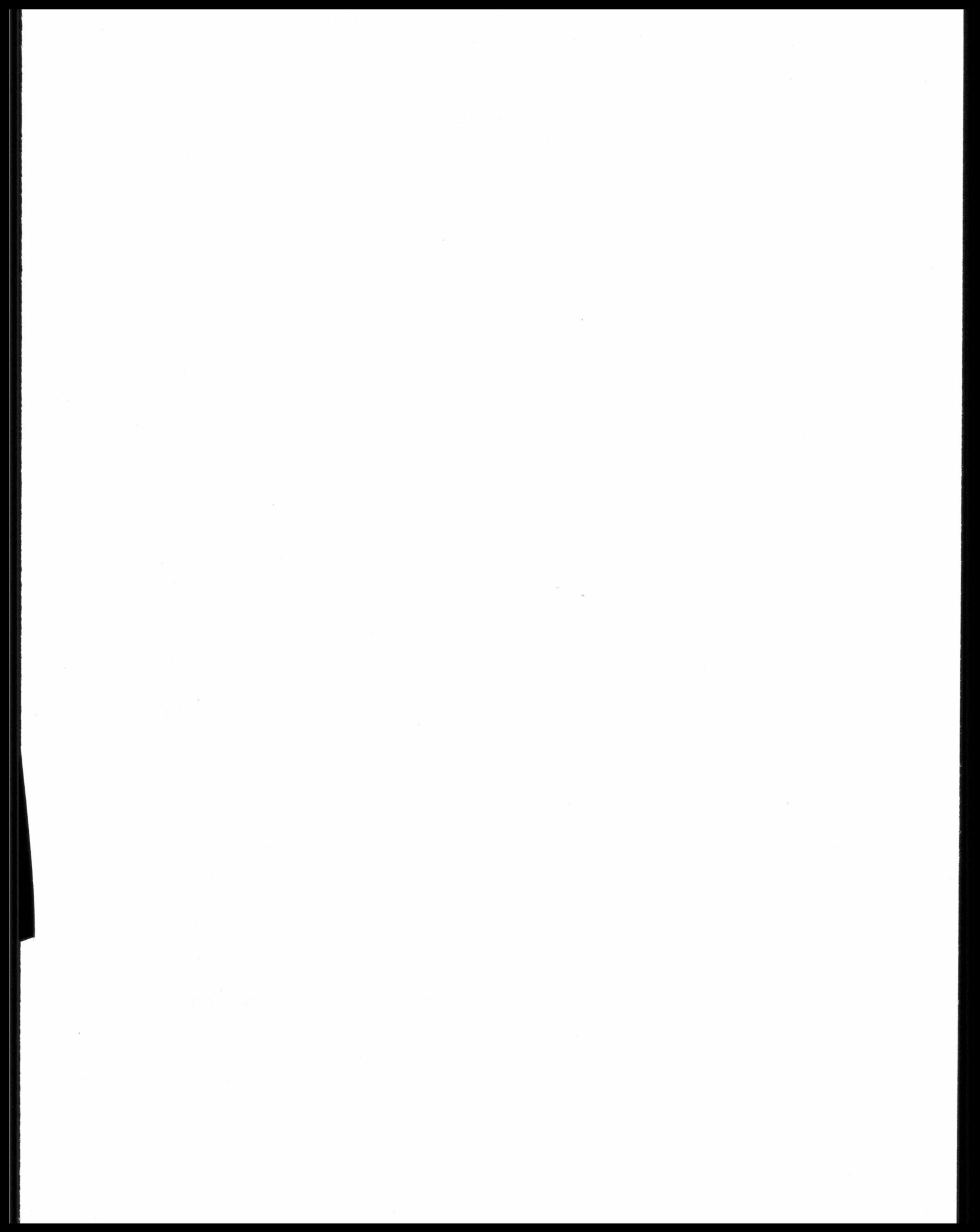


Figure 3: The area under the curve (AUC) for sensitivity vs. specificity of the final model, showed relative to random predictability. AUC=0.928, with a maximum value of 1 and significance at values above 0.75.





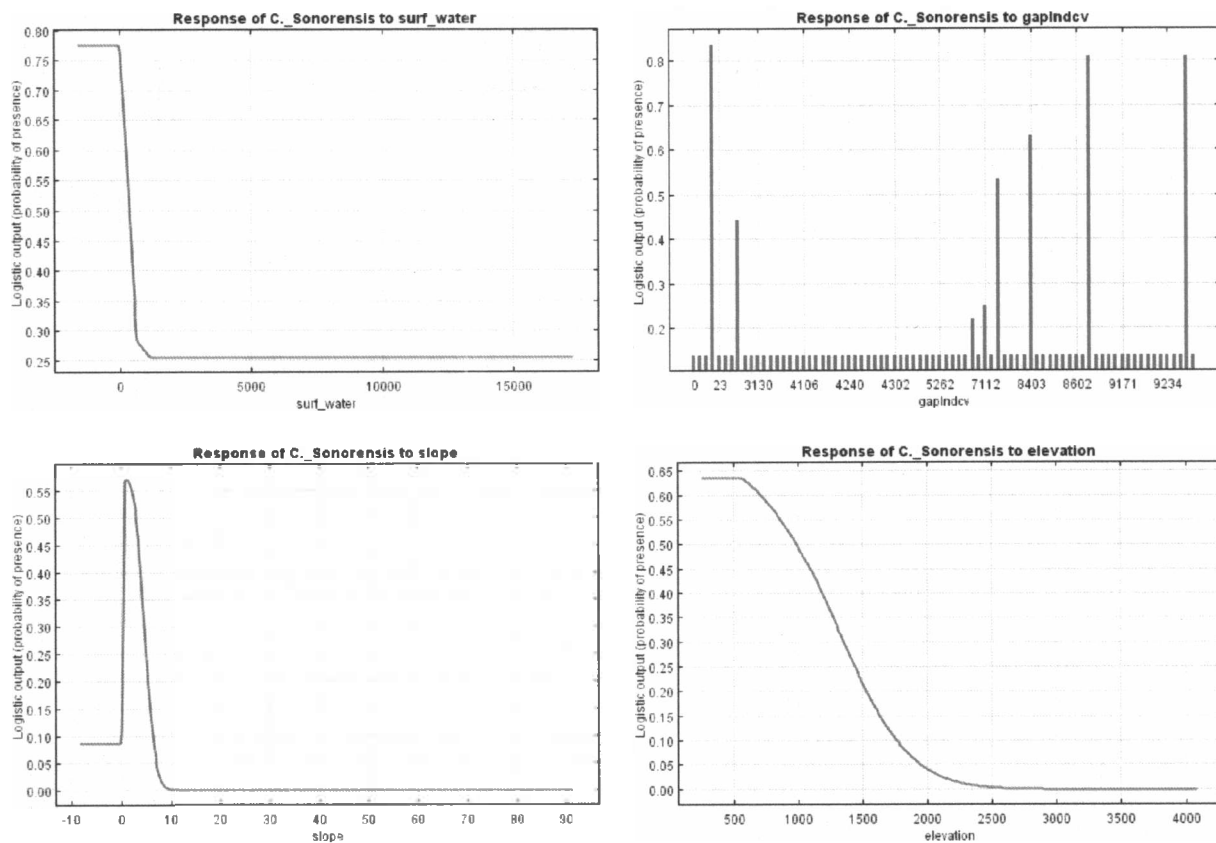


Figure 5: Response curves for the four environmental variables included in the final model of *C. sonorensis* habitat suitability. Independent variables are both continuous (distance to surface water, slope, elevation) and categorical (land cover). Metadata is available in Appendix A.