

**GUNNISON'S PRAIRIE DOG CONSERVATION PLAN:
ADDENDUM TO THE
WHITE-TAILED AND GUNNISON'S PRAIRIE DOG
CONSERVATION STRATEGY**



Western Association of Fish and Wildlife Agencies
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RECOMMENDED CITATION

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INTRODUCTION

The Gunnison's prairie dog (GPD; *Cynomys gunnisoni*) plays an important role as a keystone species in maintenance of sage-steppe and prairie scrub ecosystems. Within the last century this species has declined in distribution and abundance throughout its range (Seglund et al. 2006). Factors contributing to this decline include, but are not limited to, habitat loss, poisoning, and sylvatic plague.

In 2004, GPD long-term viability was questioned by a petition to list the species under the Endangered Species Act (ESA; Forest Guardians 2004). The petition cited habitat loss/conversion, shooting, disease, a history of eradication efforts, and inadequate federal and state regulatory mechanisms as threats to the long-term viability of the species. Prior to the petition being submitted, state wildlife agencies initiated the development of a multi-state Conservation Assessment to evaluate the range-wide status of the GPD and evaluate impacts to the species.

After completing the Assessment in 2006 (Seglund et al. 2006), the states and federal cooperators developed a Conservation Strategy (Western Association of Fish and Wildlife Agencies [WAFWA] 2006) for both the GPD and the white-tailed prairie dog (WTPD; *C. leucurus*). The Conservation Strategy provided management and administrative guidelines to assist in the development of state management plans for GPDs and associated ecosystems. The objective of state and federal agencies involved in GPD management under the Strategy is to conserve and maintain viable prairie dog populations and the sage-steppe and prairie scrub ecosystems they inhabit. This effort contributed to a U.S. Fish and Wildlife Service (USFWS) negative 90-day finding in 2006 for the GPD listing petition (USFWS 2006). This Conservation Plan is an addendum to the Conservation Strategy and describes specific activities state wildlife agencies will include in individual state plans to improve prairie dog conservation and management.

This effort is being led by WAFWA, working through its Prairie Dog Conservation Team (PDCT; Appendix A), which works with all prairie dog species, and the species-specific White-tailed and Gunnison's Prairie Dog Working Group (WTGWG; a committee under the PDCT). Actions under the Conservation Strategy and Conservation Plan are designed to: 1) promote conservation of the GPD and its habitat; 2) reduce the risk of overutilization of GPDs for commercial, recreational, scientific, or educational purposes; 3) identify specific research needs; 4) manage existing regulatory mechanisms to maintain species viability; 5) reduce the risk of factors negatively impacting GPD populations; and 6) increase landowner participation in prairie dog conservation efforts and minimize impacts from possible lost management options. The Conservation Strategy and Conservation Plan recognize that population control is appropriate in certain circumstances.

The state wildlife agencies will implement the Conservation Strategy and Conservation Plan and will seek funds to enhance implementation. Planning and management proposals and actions will also be coordinated with federal agencies through the individual state management plans. The involvement of tribes, other government agencies, and private entities will be invited and their participation welcomed. Effective conservation of GPDs and their habitat under the Conservation Strategy and Conservation Plan will depend on cooperation of all groups, including private landowners, to the extent they wish to be included. Cooperators recognize the importance of rural livelihoods and activities (e.g., ranching, outdoor recreation), and voluntary participation by private landowners in habitat identification, enhancement, and conservation as key to the success of the Conservation Strategy and Conservation Plan.

The purpose of the WTGWG is to assist with and coordinate activities of the states and other working group members, including: 1) standardizing data collection and improving monitoring and inventory techniques to determine range-wide status, 2) recommend conservation action for protection and maintenance of the species as well as evaluating the economic impacts to local populations, and 3) identifying research needs and helping to obtain funds to implement projects. WTGWG members may be assigned to various technical committees as information or other needs (e.g., review of materials) arise. Each state wildlife agency PDCT member is responsible for coordinating the Conservation Strategy and Conservation Plan activities within their respective state.

CONSERVATION STRATEGY

The Conservation Strategy identifies both short and long-term objectives, and sets time frames for completing activities. It incorporates a range-wide view for long-term species persistence and an ecosystem management approach for habitat conservation.

Although the Conservation Strategy focuses on WTPD and GPD conservation, participants recognize, because these prairie dogs are considered keystone species, the risks identified for them also may affect associated sage-steppe and prairie scrub species. Initially, participants agree to direct their conservation actions toward WTPDs and GPDs, but when applicable, will work toward conservation of sage-steppe and prairie scrub habitat and associated species.

The Conservation Strategy has 9 objectives, with activities listed under each objective, for conserving WTPDs and GPDs across their range. The objectives allow cooperators to manage prairie dog populations in a manner that ensures long-term viability while also maintaining management flexibility. This Conservation Plan identifies specific activities for GPD management that will accomplish the objectives outlined in the Conservation Strategy.

Conservation Objectives

The conservation objectives outlined in the Conservation Strategy are as follows:

1. Implement the Conservation Strategy.
2. Continue participation on the Prairie Dog Conservation Team, White-tailed and Gunnison's Prairie Dog Working Group, and state work groups if formed.
3. Identify, monitor, and manage the distribution, population, and status of both species to maintain the current distribution within the normal range of biological variation.
4. Promote public outreach and education.
5. Identify, prioritize, and implement research needs.
6. Address the 5 listing factors in individual state management plans.
7. Integrate WTPD and GPD conservation strategy objectives with management and habitat objectives of other sage-steppe and prairie scrub species such as greater sage-grouse (*Centrocercus urophasianus*), Gunnison sage-grouse (*Centrocercus minimus*), ferruginous hawk (*Buteo regalis*), black-footed ferret (*Mustela nigripes*), burrowing owl (*Athene cunicularia*), kit fox (*Vulpes macrotis*), and pygmy rabbit (*Brachylagus idahoensis*).
8. Develop a detailed addendum to the Conservation Strategy.
9. Evaluate progress and accomplishments.

GOAL

The goal of this Conservation Plan is to conserve GPD populations within each state to ensure long-term population viability by maintaining the current distribution identified in the conservation assessment, within the normal range of biological variation, thereby precluding the need for regulation under the ESA. This will be achieved as states maintain occupancy across at least 75% of the geographic range of the species within the state.

CONSERVATION ACTIVITIES

Priority issues have been identified and are listed below in order of their future, potential negative impacts on GPDs. Conservation activities were developed to address these impacts.

Priority issues and associated conservation activities

- A. *Plague*. Develop management actions to mitigate plague outbreaks. These actions may include dusting, translocation, closures, land protection, predator control, and increased monitoring.
- B. *Range condition (i.e. non-native species, altered fire regimes, drought, juniper encroachment)*. Management actions may include habitat manipulation (i.e., chaining, initiating fire regimes, reseeding native grasses, and cheat grass eradication).
- C. *Chemical control*. Management actions may include developing conservation easements or non-lethal control options (translocation, public education, green barriers)
- D. *Shooting*. Management actions may include reviewing regulations, implementing closures, monitoring take, and educating the public.
- E. *Oil/gas development*. Management actions are currently underway and will be ongoing. These include tracking impacts to colonies when development is on or near them. State agencies will continue commenting on development plans.

In addition to the activities above and those outlined in the Conservation Strategy, this Conservation Plan will provide additional specific activities and targets to accomplish Objectives 3, 4, 5, 6, and 9 in the Strategy. The activities listed below are intended to mitigate potential impacts to GPDs over the life of the Conservation Plan. Some activities have already been initiated and others will be activated by a trigger.

Identify and monitor the distribution and status of the species (Conservation Strategy Objective 3)

- A. *Determine current status and distribution*. Estimating the current status and distribution of the GPD is difficult, and currently the amount of occupied habitat range-wide is unknown (Seglund et al. 2006). A Predicted Range Model (PRM) was developed as a potential spatial depiction of the range of the GPD; this was accomplished using Geographic Information Systems (GIS) datasets and literature related to habitat characteristics for the GPD (Seglund et al. 2006).
 - 1. Complete an initial GPD survey in each state during 2007 to determine the rate of occupancy within potential GPD habitat (see Appendix B).
 - a. All state wildlife agencies will use the methodology outlined in the occupancy protocol (Appendix B).
- B. *Monitoring GPD populations*. Long-term monitoring of GPD populations will be conducted in all states to establish population trends and monitor status.

1. Through monitoring, the states will identify a baseline occupancy rate and ultimately, a GPD population trend over several sampling periods. It was determined by the PDCT that 700 plots will be sampled range-wide to detect trends in the population.
2. Develop standard reporting methods among the contributors as identified in the Prairie Ecosystem MOU (Appendix C).
3. Actions (see below *a.*) will be triggered when/if a 40 % (95% CI) range-wide occupancy decline is detected between surveys (i.e. 3 years; short-term trigger). A long-term trigger for action will also be identified. However, since process variation is unknown, this will not take place until after 3 sample periods (i.e. 6 years) have occurred. This timeframe will provide sufficient data to identify a long-term trigger. This trigger will be retroactive; if a certain percent decline is detected, management actions will be initiated immediately.
 - a. Actions Triggered: Within 1 year of reaching the trigger, a course of action, on a state-by-state basis will be developed by the PDCT and presented to WAFWA Directors for implementation. Though the range-wide trend is being monitored, the species may only be affected in distinct geographic areas. However, all states will support actions taken even if the area in decline is not within their state boundaries. Support may include providing funding or personnel.
 - b. Surveys will be conducted annually until the trigger is reset.

C. Identify suitable GPD habitat. The PRM that was developed for the GPD Conservation Assessment (Seglund et al. 2006) identified the gross potential range for GPDs. States will use the PRM as a base to develop more refined models that can then be ground-truthed.

Promote public outreach and education (Conservation Strategy Objective 4)

- A. All interested parties (e.g. landowners, non-governmental organizations) will have the opportunity to participate in GPD state management plan development. Methods and extent of participation may vary from state to state (e.g. working group membership, public comment period).
- B. States will make personal contacts and develop working relationships with owners of key habitat tracts, and any other landowner who expresses an interest in the GPD and associated habitat conservation.
 1. Develop strong partnerships with Natural Resources Conservation Service (NRCS) Range Conservationists, to enable using existing NRCS programs for landowner incentives.

- C. Development and dissemination of public informational materials and programs to educate interested parties on GPD natural history, importance of their role as a keystone species, and threats to their populations and associated habitats.

Identify, prioritize, and implement research needs (Conservation Strategy Objective 5)

The USFWS (2006) determined that although none of the 5 listing factors were threats to the continued existence of the GPD, more information was needed on 3 of the 5 listing factors. More information is needed to allow better management regarding: land use practices affecting GPD habitat and distribution, the effects of plague, and effectiveness of current regulatory mechanisms. Additional information on these factors will aid in the design of management strategies to alleviate additive stresses during difficult environmental conditions and provide information on when, how, and to what extent control measures should be used.

The identified research needs will not necessarily be implemented or funded at the individual state wildlife agency level. Rather, these will serve as recommendations to WAFWA partners and researchers and will be financially supported by state wildlife agencies as funding and staff are available. However, state wildlife agencies are encouraged to share research responsibilities so efforts are not duplicated. The WTGWG will pursue obtaining funds for the highest priority research needs.

- A. *Disease*. The effect of plague on long-term viability of GPDs is unknown. Currently, no techniques are available for large-scale effective control or management because the ecology of plague differs between habitats, populations, and prairie dog species. Flea control methods are costly and labor intensive, but can be successfully used on a small scale (D. Biggins, USGS, pers. comm.). An integral part of managing plague and protecting GPD populations will be to understand the range-wide dynamics of plague.

1. Highest priority

- a. Continue research on the use of pesticide dusting for flea control as a management tool. GPD colonies with plague have been found to have both a higher percentage of burrows infested with fleas and a greater number of fleas per infested burrow than plague free colonies, indicating that fleas may drive the cycle (Heller 1991).
- b. Further examine conditions (e.g., weather) under which plague outbreaks are likely.
- c. Evaluate ramifications of plague for long-term persistence of GPD populations at a landscape scale.
- d. Examine recovery rates and population dynamics of infected colonies.
- e. Examine the feasibility of using translocations to augment local prairie dog populations reduced by plague outbreaks.
- f. Continue research to develop an oral plague vaccine that can be economically dispersed over large areas occupied by GPDs.
- g. Determine what happens to plague between epizootics (maintenance mechanisms).

- h. Determine the role of associated mammals in maintenance and transmission of plague.
 - i. Determine the mechanisms by which plague is spread between GPD colonies.
 - j. Determine the long term potential for plague to preclude attainment of GPD conservation objectives.
 2. Medium priority
 - a. Model GPD metapopulation dynamics and viability in the presence of plague.
 - b. Determine the mechanisms by which GPD colonies in the Aubrey Valley Complex, Arizona remain free of plague.
 - c. Determine whether inbreeding depression occurs in recovering colonies.
- B. *Habitat loss.* Studies should be conducted to identify habitat characteristics required to maintain viable GPD populations and to address the direct and indirect effects of land conversions on GPDs.
 1. Highest priority
 - a. Determine the effects of timing and intensity of common grazing practices on GPD habitat use.
 - b. Determine the effects of fragmentation, and development of barriers due to urbanization and agricultural development, on dispersal and maintenance of colonies.
 - c. Evaluate changes in distribution and population densities at sites prior to, during, and after oil and gas development.
 - d. Evaluate colonization rates after oil/gas wells are removed.
 - e. Evaluate the effects of vibroseis on GPDs.
 2. Medium priority
 - a. Determine the effects of agricultural land conversions on population densities, reproductive output, and long-term viability.
 - b. Determine the spatial and temporal effects of fire on GPD colonization rates and re-colonization rates.
 - c. Determine differences between non-native annual grasses and native plants in effects on population trends, reproductive output, and viability over the long-term.
 - d. Monitor impacts of range restoration treatments, such as green-stripping with forage kochia, on GPD populations.
 - e. Examine the genetic structure of GPD metapopulations.
- C. *Regulatory mechanisms.* State and federal agencies will review and evaluate current laws and regulations regarding GPDs. State wildlife agencies and federal agencies will cooperate on development of new Resource Management Plans that address species-specific needs of

GPDs and their habitat with regard to oil and gas development, livestock grazing, poisoning, shooting, and road development. Standardized range-wide monitoring and management strategies for GPD colonies will be developed and implemented to measure and potentially mitigate the impacts of disturbances. In addition, research addressing many of the issues associated with GPD biology, ecology, and response to disturbances will be implemented and attempts will be made to coordinate with private land owners to protect existing colonies and explore possibilities for colonization on private properties. Mitigation options for development in areas currently occupied by GPDs and design and implementation of translocation programs will be identified for consideration by the appropriate entities.

1. Highest priority

- a. Each state will analyze existing regulations and their impacts on GPD conservation. The analysis will identify specific regulatory needs necessary to conserve the GPD.

D. Shooting. Shooting, unlike plague, is a manageable factor impacting prairie dogs. State wildlife agencies will re-evaluate their current regulatory authorities and measures to ensure appropriate regulated take of GPDs. States will consider implementing seasonal closures when females and pups are most vulnerable (1 April-15 July). States will develop monitoring techniques to assess the impacts of shooting and the potential need for regulations to limit take. Each state will develop a means of monitoring GPD harvest.

1. Medium priority

- a. Development of an appropriate monitoring technique to enable managers to make shooting sustainable over time and avoid extinctions of local populations.
- b. Studies comparing exploited and non-exploited GPD populations will be conducted. Analysis will include effects on social interactions, foraging, distribution, emigration, population trends, and reproductive output. Studies will be conducted on a large scale over an extended time period to accurately evaluate the effects of recreational shooting.
- c. Studies will be conducted that evaluate different levels of shooting pressure on GPD populations. This will provide information to help manage harvest levels and timing to protect populations.

E. Chemical Control. Ultimately, poisoning must be managed by state wildlife agencies or state departments of agriculture if regulation of GPD take is necessary. Development of incentive programs to motivate private landowners to maintain GPD colonies on their lands will be explored. Translocation to supplement existing colonies, create new colonies, and/or move individuals from colonies threatened with imminent destruction could be incorporated into management plans to help maintain or recover population densities.

1. Medium priority

- a. Evaluate the use of translocation as an alternative to poisoning. Specifically, evaluate different methods of trapping, survival of translocated individuals, and feasibility of large vs. small scale translocation efforts.
- b. Examine the ability of GPD populations to rebound after use of poisons on colonies.
- c. Develop non-lethal options for controlling GPDs.

F. Drought. Climate conditions cannot be managed directly, but other effects that might exacerbate potential drought impacts can be evaluated and managed, if necessary. GIS data layers could be used to rate sites on their ability to sustain GPDs during times of drought, based on composition of vegetation and location of the habitat. For instance, areas composed of native vegetation could be considered less at risk than areas dominated by cheatgrass or other vegetation less suitable to GPDs. This could help land managers focus on high risk areas through a variety of strategies, such as evaluating the timing and intensity of grazing to promote forb and perennial grass production, controlling invasive weeds, and restoring the historical density of woody species. Also, managers could work to alleviate other impacts, such as shooting, which might affect the amount of time a GPD spends foraging, to minimize negative impacts of drought.

1. Medium priority

- a. Monitor GPD populations during various environmental conditions over a significant part of the range
- b. Examine land use practices and their ability to influence GPD responses to environmental changes
- c. Research population dynamics under drought conditions
- d. Study the effects of grazing in areas occupied by GPD during drought years

Address the 5 listing factors in individual state management plans (Conservation Strategy Objective 6)

The following activities may be modified pending research outcomes.

- A. All 4 states will implement the Plague Protocol (Appendix D) or other existing plague monitoring methodology, in cooperation with private landowners and other entities in order to monitor for plague and to document and respond to significant sylvatic plague events.
- B. Develop adequate management approaches to mitigate the impacts of plague.
- C. Compatible with the goals and objectives in the state management plan, develop regulatory authority for conservation of prairie dogs by establishing, through law or regulation, the ability of the state wildlife agency to limit or prohibit take by shooting. Seasonal or year-long closures to control take may be necessary to maintain the objectives in the state management

plan. Individual states will retain the option to close the season year-long or seasonally on all lands under their jurisdiction within the state, or only on public lands. Institution of seasonal or yearlong closures on tribal lands will be done only under the authority of the respective tribal governments. Seasonal closure during the whelping and dependent young period, 1 April through 15 July, to address a decline in occupied habitat will be an option that can be evaluated annually. If an individual state has the regulatory authority to close the shooting season, it may choose to apply the closure or restriction if shooting appears to be an imminent threat to long-term viability of any segment of the statewide population.

To be most effective, state programs will include the following elements:

1. Annual field checks and/or mail or phone surveys to collect data on harvest, hunter days per county, and hunter days/harvested animal
 2. The data collected will provide quantification of the extent of prairie dog shooting, allow extrapolation of the economic value to the state, provide data from which to judge the impact of shooting on populations, indicate where shooting regulations may be required to limit take, and the ability to conduct trend analysis.
- D. Compatible with the goals and objectives in the state management plan, acquire or maintain adequate regulatory authority for conservation of prairie dogs by establishing through law, regulation, or cooperative agreement the ability of the state to limit or prohibit take by poisoning. This authority would be invoked only if objectives in the state management plan were not being met.
- E. Develop and implement strategies to appropriately reduce conversion of GPD habitat to other land uses in each state (e.g., conservation easements).
- F. Monitor habitat conversions and cumulative effects over the range on a five-year basis in cooperation with NRCS and state agricultural statistics agencies.
- G. Document reduced occupancy rates or numbers of acres lost, and analyze the potential of the loss to impact the ability of the state to maintain or increase towards target objectives.
- H. Develop appropriate management responses if monitoring indicates habitat conversion trends that appear to significantly threaten maintenance of, (if target objectives have been met), or progress toward, prairie dog objectives.
- I. Oil and gas development should be designed to minimize adverse impacts on existing GPD colonies and potential habitat. To assess impacts at proposed sites, GPD occupied and potential habitat should be documented prior to development. The minimal analysis will include mapping of suitable and occupied habitat, use of GIS to determine spatial distribution of these areas, estimates of local population densities, and evaluation of dispersal potential

between suitable habitat patches within each complex (e.g. between colonies). Baseline information will help determine whether the loss of occupied and suitable habitat due to resource extraction activities could be mitigated by managing other suitable habitat within a proposed project site and/or avoiding suitable and occupied habitat entirely and allowing development only in habitat not suitable for GPD occupation. In addition, project design of oil and gas facilities in and adjacent to occupied and suitable habitat should include location of wells and roads outside of these areas, consideration of directional drilling when wells are proposed within suitable and occupied habitat, timing restrictions of vehicle travel to periods when GPDs are less active, and regulation of type of vehicle traffic. Also, because knowledge of the effects of resource extraction on GPD populations is limited, monitoring should be conducted at sites before, during, and after development. This will include monitoring vegetation changes after wells are constructed and when they are removed. Finally, enforcement of well reclamation should be improved.

J. Grazing effects

1. Develop grazing management practices to maintain vegetation on both upland and riparian sites. Emphasize maintenance of native plant species and natural re-vegetation. Reseed disturbed and burned areas using native, locally adapted plant species, where appropriate.
2. Where appropriate, institute the use of mechanical, chemical, and/or biological methods of weed control to eradicate noxious weeds.
3. Incorporate fire, drought, flooding, and prescribed land treatments into livestock management practices.

Evaluate progress and accomplishments (Conservation Strategy Objective 9)

Progress will be evaluated based on completion of the following items by the dates listed.

- A. Develop and implement a GPD management plan within each state (due December 2007).
 1. All interested parties (e.g. landowners, non-governmental organizations) will have the opportunity to participate in GPD state management plan development. Methods and the extent of participation may vary from state-to-state (e.g. working group membership, public comment period).
- B. Complete an initial GPD inventory in each state to determine the rate of occupancy within potential GPD habitat (due December 2007).

- C. Develop monitoring methods comparable across the 4 states and monitor occupied habitat and distribution approximately every 3 years beginning in 2007.
- D. Develop standard reporting methods among the contributors as identified in the Prairie Ecosystem MOU (Appendix C; due January 2007).
- E. Make personal contacts and develop working relationships with owners of key habitat tracts, and any other landowner who expresses an interest in the GPD and associated habitat conservation (a summary will be provided to the WTGWG annually at the PDCT meeting).
 - 1. Develop strong partnerships with Natural Resources Conservation Service (NRCS) Range Conservationists. Work with them to use existing NRCS programs for landowner incentives.
- F. Develop and disseminate public informational materials and programs to educate interested parties on GPDs and their associated habitats (a summary will be provided to the WTGWG annually at the PDCT meeting).
- G. Identify and prioritize research needs (completed July 2006).
 - 1. The WTGWG will investigate obtaining funding for identified research needs (this will be discussed annually at the PDCT meeting).
- H. All 4 states will implement the Plague Protocol (Appendix D) or other existing plague monitoring methodology, in cooperation with private landowners and other entities in order to monitor for plague and to document and respond to significant sylvatic plague events (due 2007).
- I. Develop adequate management approaches to mitigate the impacts of plague and include in state management plans (due December 2007).
- J. Each state will analyze existing regulations and their impacts on GPD conservation. The analysis will identify specific regulatory needs necessary to conserve the GPD and be included in the individual state management plans (due December 2007).
- K. Develop regulatory authority for conservation of prairie dogs by establishing, through law or regulation, the ability of each state wildlife agency to limit or prohibit take by shooting (due December 2007).
- L. Acquire or maintain adequate regulatory authority for conservation of prairie dogs by establishing through law, regulation, or cooperative agreement the ability of the state to limit or prohibit take by poisoning (due December 2007).

- M. Create/designate a coordinator position and/or other positions as needed in each state to conduct or supervise GPD conservation (all states have a full or part time coordinator).
- N. Develop and implement strategies to monitor and reduce conversion of GPD habitat to other land uses in each state. Include these in the individual state management plans (due December 2007).
- O. Monitor habitat conversions and cumulative effects over GPD range on a 5-year basis in cooperation with NRCS and state agricultural statistics agencies (beginning in 2007).
- P. Develop appropriate management responses if monitoring indicates habitat conversion trends that appear to significantly threaten prairie dog populations. Include these in the individual state management plans (due December 2007).
- Q. Develop, advocate, and promote best management practices for oil and gas development designed to minimize adverse impacts on existing GPD colonies and potential habitat (due December 2007).
 - 1. To assess impacts at proposed sites, GPD occupied and potential habitat should be documented prior to and post development.
- R. Address grazing management practices in individual state management plans (due December 2007).
 - 1. Emphasize maintenance of native plant species and natural re-vegetation. Reseed disturbed and burned areas using native, locally adapted plant species, where appropriate.
 - 2. Where appropriate, institute use of mechanical, chemical, and/or biological methods of weed control to eradicate noxious weeds.
 - 3. Incorporate fire, drought, flooding, and prescribed land treatments into livestock management practices.

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Appendix A. List of state wildlife agencies and identified members of the Prairie Dog Conservation Team.

State Wildlife Agency	PDCT Members	Prairie Dog Species in the State
Arizona Game and Fish Department	Bill Van Pelt, Nongame Birds and Mammals Program Manager Jared Underwood, Small Mammal Coordinator	BTPD (extirpated) GPD
Colorado Division of Wildlife	Eric Odell, Wildlife Conservation Biologist (BTPD) Amy Seglund, Wildlife Conservation Biologist (GPD) Pamela Schnurr, Wildlife Conservation Biologist (WTPD)	BTPD GPD WTPD
Kansas Department of Wildlife and Parks	Mike Mitchener, Wildlife Section Chief	BTPD
Montana Fish, Wildlife, and Parks	Allison Puchniak, Native Species Biologist	BTPD WTPD
Nebraska Game and Parks Commission	Mike Fritz, Natural Heritage Zoologist	BTPD
New Mexico Department of Game and Fish	Jim Stuart, Nongame and Endangered Species Mammals Specialist	BTPD GPD
North Dakota Game and Fish Department	Patrick Isakson, Nongame Biologist	BTPD
Oklahoma Department of Wildlife Conservation	Julianne Hoagland	BTPD
South Dakota Game, Fish, and Parks	Silka Kempema, Wildlife Biologist	BTPD
Texas Parks and Wildlife Department	John Young, Mammalogist	BTPD
Utah Division of Wildlife Resources	Kevin Bunnell, Mammals Program Manager Anthony Wright, Sensitive Species Biologist (GPD) Brian Maxfield, Sensitive Species Biologist (WTPD)	GPD WTPD UPD
Wyoming Game and Fish Department	Martin Grenier, Nongame Mammal Biologist	BTPD WTPD

Note: The state wildlife agency representatives may change over time. This list is current at time of printing.

Appendix B. Protocol for Conducting Prairie Dog Occupancy Surveys

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Prepared for

WTPD and GUPD Working Group

February 2007

Introduction

The White-tailed (*Cynomys leucurus*; WTPD) and Gunnison's Prairie Dog (*C. gunnisoni*; GUPD) Conservation Plan (WAFWA 2007) required the development and use of an objective, repeatable estimation technique to measure the response of WTPD and GUPD populations to factors affecting their viability. Techniques used to evaluate prairie dog populations have relied on delineating colony boundaries based on burrow distribution. However, WTPD and GUPD colony boundaries can be difficult to map with distribution and activity levels within boundaries extremely variable. The end result of mapping is therefore a subjective effort by investigators who rely on their best estimate by using topographic features or breaks in habitats to delineate boundaries. In addition, individual burrow activity is not assessed, resulting in both active and inactive areas included in estimates of occupied habitat. The consequence of mapping both active and inactive areas is an inaccurate estimation of occupied habitat.

In 2002, Colorado embarked on an effort to develop an objective technique to monitor WTPD and GUPD populations. Aerial surveys using the line intercept methodology had been developed for estimating occupied area by black-tailed prairie dogs (*C. ludovicianus*). Thus this was the first method investigated to determine if it could be successfully used for WTPD and GUPD. After conducting a pilot study, it was determined that the line intercept methodology significantly overestimated the lengths of GUPD and WTPD colonies compared to lengths measured on the ground. In addition, the proportions of lengths of prairie dog colonies detected by aerial crews were only weakly correlated; the crews did not consistently report finding prairie dogs in the same areas along transects. Due to the lack of correlation between aerial and ground crews, the line intercept methodology was abandoned as a viable technique to monitor WTPD and GUPD populations.

After abandoning the use of the line intercept methodology, Colorado investigated using Occupancy Modeling (MacKenzie et al. 2002) as an objective technique to monitor WTPD and GUPD. Unlike acreage estimates, measures of statistical precision and confidence intervals could be calculated for occupancy estimates. Currently Colorado is implementing Occupancy Modeling for both WTPD and GUPD within in the state. Colorado has completed one year of surveys in 2004 for WTPD and in 2005 for GUPD. Results from the surveys found WTPD occupying 24.1% (SE = 12.8) of 47,710 0.25-km² plots and GUPD occupying 7.5% (SE = 1.3) of 158,225 0.25-km² plots (Andelt et al. 2005).

Occupancy surveys have the potential to be a successful tool for establishing baseline occupancy rates for WTPD and GUPD in order to monitor changes in occupancy through time (Andelt et al. 2005, 2006a, 2006b). This manuscript was prepared to standardize occupancy surveys throughout the range of both the GUPD and WTPD. All states within the range of these species have agreed, in the Multi-state Conservation Plans, to implement an occupancy approach to monitor range-wide WTPD and GUPD population trends.

Range-wide Methodology for Occupancy Sampling for WTPD and GUPD

Defining Sampling Areas: Occupancy will be estimated by sampling 0.25 km² (0.5 km per side) quadrats. Quadrats will be randomly selected within each state boundary in areas designated as suitable WTPD and GUPD habitat. This defined area of inference within states will remain constant throughout the duration of the monitoring effort. In addition, the quadrats randomly selected to be sampled will not change unless all quadrats are disposed of and a new set of quadrats are randomly selected from the area of inference.

Suitable habitat does not necessarily mean that the habitat is occupied, rather it is defined as suitable or potentially suitable based on variables designated by a state as necessary for prairie dog colonization. States need not define their areas of inference in the same manner in order to conduct a range-wide occupancy survey. It is only necessary that the states develop the most accurate area of inference from the best available data. The area of inference may include tribal lands if the state is given permission to sample these lands, however they should be placed in a different strata since the permission to sample these lands may be removed at any time.

States may wish to include the use of stratification. Stratification is useful for:

- Interest in occupancy at subdivisions smaller than the whole state or range
- Logistical convenience (ability to sample an entire stratum quickly and with similar methods)
- Need for different methods in different areas (some strata may be more easily sampled from the ground versus the air, some strata may have very good information on prairie dog locations)
- Variance reduction (individual strata with uniform occupancy rates will increase precision)

States however do not need to stratify and in addition, stratification does not need to be the same within each state boundary in order to conduct a range-wide occupancy approach.

Below is a description of how Colorado developed their area of inference and selected quadrats to sample for both WTPD and GUPD.

Colorado - Protocol for Developing Base Maps to Overlay Quadrats

Methods

WTPD: Development of Maps and Sampling Areas: Field personnel from the Colorado Division of Wildlife, Forest Service, and the Bureau of Land Management mapped colonies of active (prairie dogs present during the last \pm 3 years), inactive (prairie dogs occurred in the area in the past but were not recently present) and unknown (prairie dogs had been active but current status was unknown) WTPD colonies on 1:50,000 US Geological Survey County maps in the summer of 2002 (Colorado Division of Wildlife 2002). These data, in addition to data on the overall range of WTPD areas were input into a GIS database by Colorado Division of Wildlife personnel. The final product included active, inactive, and unknown colonies, and the overall range of white-tailed prairie dogs in each county on 11 x 17-inch (28 x 43-cm) colored

topographic maps which contained an overlay of township, range, and sections. County extension agents, weed and pest supervisors, and Natural Resources Conservation Service, USDA Forest Service, Bureau of Land Management, and CDOW personnel reviewed and updated the sampling frame (Figure 1).

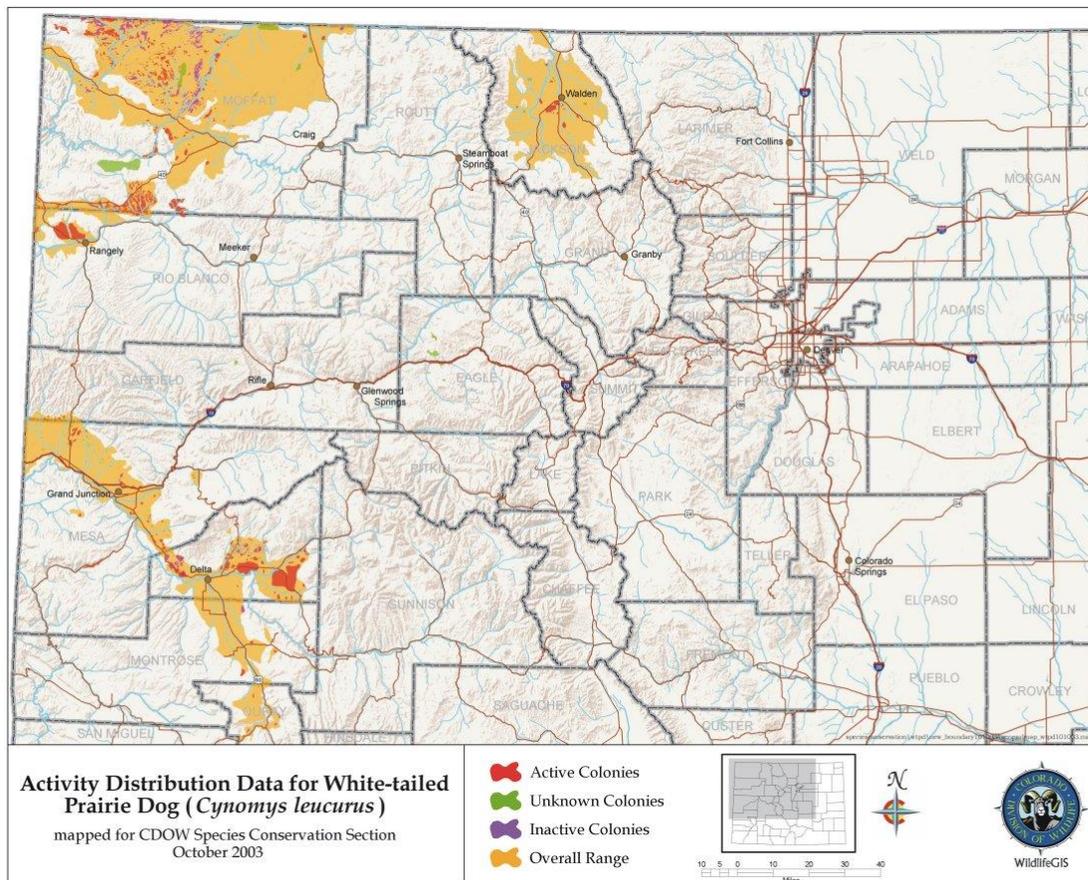


Figure 1. Range of white-tailed prairie dogs in Colorado. Three primary sampling strata consisted of Moffat and Rio Blanco counties, Eagle, Grand, Jackson, Larimer, and Routt counties, and Delta, Garfield, Mesa, Montrose, and Ouray counties.

WTPD: Selection of Quadrats: The range of WTPD in Colorado was overlaid with 1,640 x 1,640 feet (500 x 500 m) quadrats in ArcInfo using the NAD27 datum and the Zone 13 projection. Quadrats were eliminated if they occurred above 10,000 feet (3,048 m) elevation (using the 30 m digital elevation model), were on slopes >30°, or were in vegetation where WTPD do not occur. A sampling frame of 47,710 quadrats was established from which a stratified random sample of 318 quadrats was selected from 10 strata (Table 1). Three general areas were sampled: Grand Junction (GJ), North Park (NP), and Northwest (NW). Quadrats in GJ and NW were classified a priori based on Colorado Division of Wildlife GIS layers as active, inactive, unknown, or other. Quadrats in NP were classified as either unknown (active, inactive,

unknown) or other. The number of quadrats in each stratum was optimized based upon our a priori estimates of the probability (active = 0.9, unknown = 0.5, inactive = 0.1, and other = 0.05) of WTPDs being present within quadrats.

Table 1. Stratification for the sample of 318 quadrats from 10 strata of the WTPD occupancy survey in northwestern Colorado.

Strata	Stratum Population	Stratum Sample
GJ Active	1,963	20
GJ Inactive	170	12
GJ Other	11,654	55
GJ Unknown	523	9
NP Other	7,442	35
NP Unknown	462	7
NW Active	4,237	53
NW Inactive	1,278	23
NW Other	19,289	96
NW Unknown	692	8
Total	47,710	318

GUPD: Sampling Areas and Selection of Quadrats: A sampling area for GUPD was established preliminary from range maps in Armstrong (1972) and Fitzgerald et al. (1994). However, the sampling area was expanded by including areas in north-central Archuleta County, north-west El Paso County, and extreme north-east San Miguel County where colonies of GUPD were reported or where they were believed to possibly occur (Colorado Division of Wildlife 2002). Delta County, the northeastern portion of Montrose County, and the northern half of Ouray County were eliminated from the sampling area because prairie dogs in these areas are WTPD (P. M. Schnurr, Colorado Division of Wildlife, personal communication). This modified range was input in a GIS database by personnel from the Colorado Division of Wildlife. Seven strata (Figure 1) were developed based upon the overall ranges (Armstrong 1972, Fitzgerald et al. 1994) of the *zuniensis* subspecies (Ute Mountain Ute Indian Reservation, Southern Ute Indian Reservation, and remaining areas [South-West]), and the *gunnisoni* subspecies (Gunnison Valley, San Luis Valley, South Park, and South-East), and geography of Colorado. The Continental divide and other mountain ridges usually separated strata.

Longhurst (1944) reported that GUPD are probably limited to 10,000 feet (3,048 m) in elevation however, in areas with warm air currents they may be found at slightly higher elevations. Pizzimenti and Hoffman (1973) and Fitzgerald et al. (1994) reported that GUPD range in elevation from 6,000–12,000 feet (1,830 to 3,660 m) across their range. Several professionals (J. Ferguson, Bureau of Land Management; M. Threlkeld, Colorado Department of Agriculture; J. A. Capodice, Bureau of Land Management [retired]; and J. F. Cully, Kansas State University; personal communications), familiar with Gunnison's prairie dogs in Colorado, indicated that they generally are not found above 10,000 feet (3,048 m) elevation.

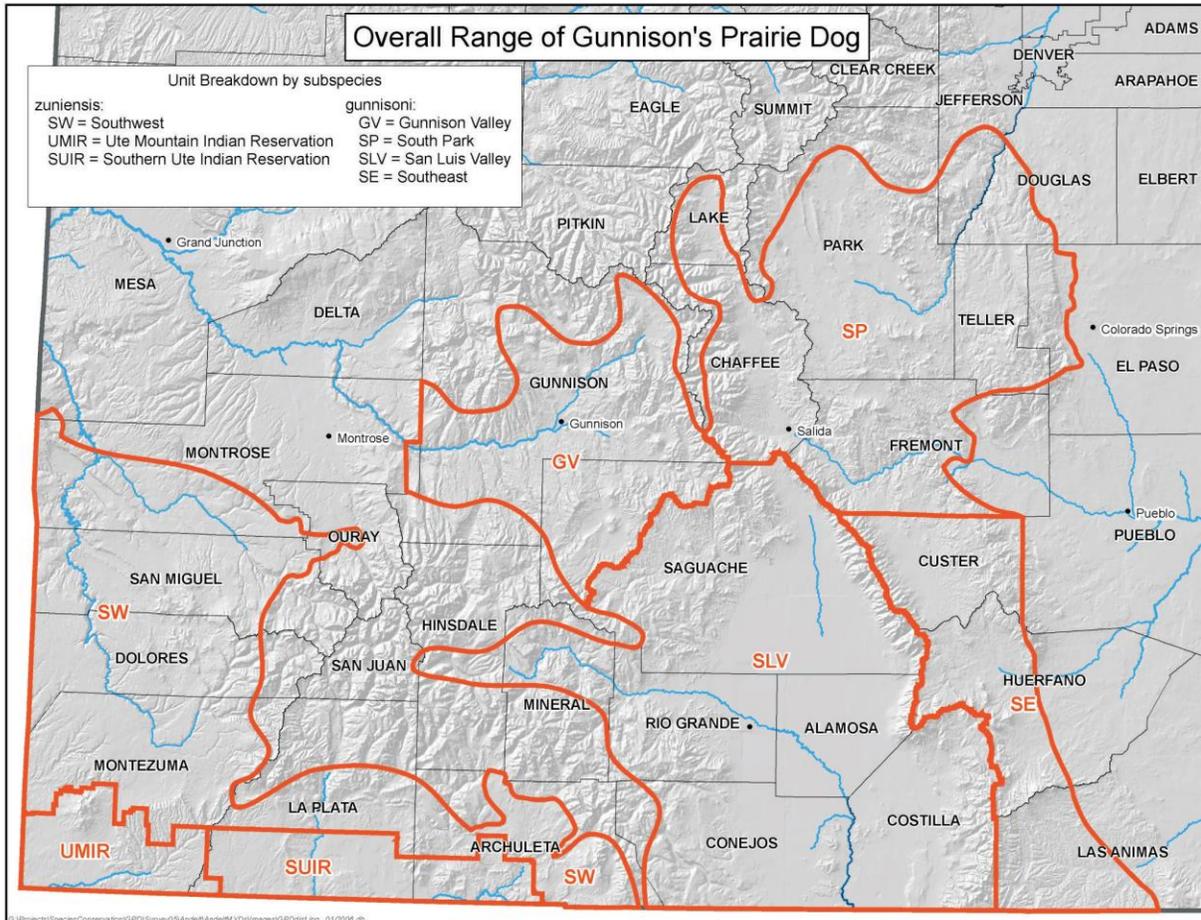


Figure 1. Strata used for sampling Gunnison's prairie dogs in Colorado during 2005.

GUPD have been described as inhabiting grasslands (Travis and Slobodchikoff 1993, Travis et al. 1997, Bangert and Slobodchikoff 2000, Perla and Slobodchikoff 2002, Girard et al. 2004), grasslands and shrub-grasslands (Cully 1997), grasslands to montane meadows (Findley et al. 1975), mountain grasslands (Lechleitner et al. 1962), valley floors to higher meadows (Longhurst 1944), and alpine meadows (Perla and Slobodchikoff 2002). The above articles and the expertise of 3 professionals (J. Ferguson, Bureau of Land Management; J. A. Capodice, Bureau of Land Management [retired]; and A. E. Seglund, Utah Division of Wildlife Resources; personal communications), familiar with GUPD, was used to further refine vegetation cover types contained in the Basin Wide Geographic Information System (GIS) as potentially occupied or unoccupied by GUPD in Colorado (Appendix 1). In addition, since GUPD are generally not found on slopes >15% (Fitzgerald and Lechleitner 1974; Lorance et al. 2002 [cited by Seglund et al. 2005]; Yazzie and Sanders 2003 [cited by Seglund et al. 2005]; J. Ferguson, Bureau of Land Management; M. Threlkeld, Colorado Department of Agriculture; J. A. Capodice, Bureau of

Land Management [retired]; and J. F. Cully, Kansas State University; personal communications) a slope layer was added to better depict the suitable habitat. The overall range of GUPD in Colorado (Figure 1) was overlaid with 1,640 x 1,640 feet (500 x 500 m) square quadrats and the Basin Wide vegetation cover types in ArcInfo (ESRI, Redlands, California) using the NAD27 datum and the Zone 13 projection. Quadrats were eliminated if all areas within quadrats were above 10,000 feet (3,048 m) elevation (30 m digital elevation model), were on slopes <15%, or were in vegetation types where GUPD are not known to occur.

Three hundred and eighty-one quadrats were randomly selected from within 7 strata where occurrence of GUPD likely varied. The number of quadrats in each stratum were optimized (Table 2) based upon a priori estimates of the probability of GUPD occurrence within quadrats (W. F. Andelt, unpublished data) using the methods described in Thompson et al. (1998). Permission to visit quadrats on the Ute Mountain Ute Indian Reservation early in the sampling process was denied. Thus, this stratum was dropped from the survey, and the original sample size was reduced to 361 quadrats.

Table 2. A priori estimates of probability of occurrence of GUPD in quadrats, number of quadrats available for sampling, optimal allocation of the sampling effort, and actual numbers of quadrats sampled for each of 7 strata in Colorado during 2005.

Strata (<i>h</i>)	Estimated Probability of occurrence	Quadrats Available (U_h)	Optimal Allocation of Quadrats to Sample	Quadrats Sampled (u_h)
Gunnison Valley	0.03	14,178	20	20
South-East	0.03	15,543	21	21
San Luis Valley	0.05	47,143	83	83
South Park	0.05	27,297	48	47
Southern Ute Indian Reservation	0.25	9,823	34	34
Ute Mountain Ute Indian Reservation	0.10	7,600	20	0
South-West	0.25	44,241	155	153
Totals		165,826	381	358

Sampling of Quadrats

To locate quadrats on the ground, UTM locations of the 4-corners of a quadrat will be downloaded from ArcInfo shape files into GPS units. In addition, topographic maps (11 x 17 inch (28 x 43-cm) and land management maps (1:100,000) showing the location of quadrats will be provided to observers to assist in locating quadrats.

Quadrats will be visited 2 times during periods when prairie dogs are most active. For Colorado, these activity periods run from late March through mid-July for WTPD and late March through mid to late August for GUPD. Other states seasonal duration of sampling may differ due to

elevation and latitudinal differences. Two visits to quadrats will be attempted to determine the detection probability however, limitations due to personnel, funding, and weather may result in areas being surveyed a single time. States will prioritize non-detection sites for revisit and those sites with a positive detection on the first visit as a lower priority for a second visit.

Two visits to a quadrat must be completed within 7 days so as to minimize violating the assumption of a closed population. To avoid observer bias and minimize possible independence violations (more likely to redetect a species once it has been detected due to prior knowledge), different observers should visit the quadrat on each of the two occasions. However, if only one technician is hired to conduct surveys, it is recommended that a supervisor or second observer visit a subset of the plots. Quadrats should be sampled unless winds are greater than 23 mi/hour or there is moderate to heavy rainfall.

Visual observations of a prairie dog are the only acceptable method that counts as a positive detection. Because auditory detections are hard to pinpoint with regards to exact location of the calling animal, this type of detection cannot be used since detections need to be confirmed within a quadrat. Scat samples are also not acceptable as the age of the scat is too difficult to pinpoint without an in depth analysis.

After arriving at a quadrat corner, if an observer detects a prairie dog they do not need to visit all four corners of the plot. If the observer arrives and no prairie dogs are detected in the quadrat, they must conduct 5 minute observations at each of the four corners of the plot until they detect a prairie dog or until all four corners have been visited. If as walking between corners a prairie dog is detected you can discontinue the survey of that plot.

Data recorded for each study quadrat will include the name of the individual conducting the sampling, date, quadrat number, time spent at quadrat, and UTM coordinates of the southwest corner of the quadrat (Appendix 2). At each plot, the observer will record air temperature and wind speed averaged over 10 seconds.

During sampling of quadrats, observations of other important species such as ferruginous hawks (*Buteo regalis*), burrowing owls (*Athene cunicularia*), Mountain plovers (*Charadrius montanus*) and kit fox (*Vulpes macrotis*) can be recorded. Note that private landowners in Colorado were not informed that information on the occurrence of these species were to be collected beforehand. Some landowners later expressed concern about this oversight. We recommend that data collection be limited only to those species that landowners have specifically approved.

Estimating Occupancy of WTPD or GUPD Quadrats from Aircraft

To locate quadrats from the air, a GPS unit will be attached to a laptop computer that contains an appropriate mapping program. The coordinates for the 4 corners of each grid quadrat are entered in the program and overlaid on a topographic map. The track function can be used to show the position of the airplane relative to each quadrat and saved for later reference. The airplane is

flown at an elevation of about 100 m above ground and 3 passes spaced across each quadrat are completed. The pilot and observer both watch for prairie dogs.

Statistical Analyses

Data will be input into an access database and the analysis will be conducted by Colorado. Occupancy models (MacKenzie et al. 2002) will be fit to the observed encounter histories for WTPD and GUPD with program MARK (White and Burnham 1999) with model selection by information-theoretic methods (Burnham and Anderson 2002). MacKenzie et al.'s model estimates the probability of detection (p) during a single visit and the probability of occupancy (Ψ) based on multiple visits to quadrats. Thus, this model corrects for "false negatives", i.e., quadrats where no prairie dogs are observed, but where prairie dogs actually exist. The logit link will be used in all models to relate covariates to detection and occupancy probabilities.

Quadrat-specific covariates that will be collected to improve the estimate of occupancy probability for each quadrat include: average temperature, wind speed, starting time, and Julian date. Elevation of the quadrat and elevation squared have been incorporated as covariates to improve prediction of occupancy rates for WTPD and GUPD in Colorado and will be included in the range-wide sampling effort. If states wish to include additional covariates that they think may improve the estimate of occupancy probability they can include them in their data collection efforts.

Occupancy estimation for entire sampling frame in Colorado: Model selection results placed almost all weight on one model for both WTPD and GUPD, so model averaging was not required. However, quadrat-specific covariates greatly improved prediction of occupancy rates for both species, so a complex procedure was required to estimate occupancy rates for all quadrats in the sampling frame. For the minimum AICc model with r quadrat-specific covariates, the fitted model was

$$\hat{\Psi}_i = \frac{\exp\left(\sum_{k=0}^r \hat{\beta}_k x_{ki}\right)}{1 + \exp\left(\sum_{k=0}^r \hat{\beta}_k x_{ki}\right)},$$

where the r covariate values for observation i are $x_{1i}, x_{2i}, \dots, x_{ri}$, and $x_{0i} = 1$. The estimates from Program MARK are the intercept ($\hat{\beta}_0$) and r slope parameters ($\hat{\beta}_1, \hat{\beta}_2, \dots, \hat{\beta}_r$). The number of quadrats estimated to be occupied in stratum $h = 1, \dots, H$ ($H = 6$ for GUPD, 10 for WTPD) with the minimum AICc model that included r covariates was computed as the sum of the estimated probability of occupancy of each quadrat, $\hat{N}_h = \sum_{i=1}^{U_h} \hat{\Psi}_i$, where U_h is the number of quadrats in the population of stratum h . The total number of occupied quadrats for all strata was

estimated as $\hat{N} = \sum_{h=1}^H \hat{N}_h$. The variance of \hat{N}_h was estimated as the sum of the estimated variance-covariance matrix of the $\hat{\psi}_i$, $i = 1, \dots, U_h$, where

$$\text{V}\hat{\text{a}}\text{r}(\hat{\psi}_i) = [\hat{\psi}_i(1 - \hat{\psi}_i)]^2 \left[\sum_{k=0}^r x_{ki}^2 \text{V}\hat{\text{a}}\text{r}(\hat{\beta}_k) + \sum_{k'=0, k' < k}^{k-1} 2x_{ki}x_{k'i} \text{C}\hat{\text{o}}\text{v}(\hat{\beta}_k, \hat{\beta}_{k'}) \right],$$

and

$$\text{C}\hat{\text{o}}\text{v}(\hat{\psi}_i, \hat{\psi}_j) = \hat{\psi}_i(1 - \hat{\psi}_i)\hat{\psi}_j(1 - \hat{\psi}_j) \left[\sum_{k=0}^r x_{ki}x_{kj} \text{V}\hat{\text{a}}\text{r}(\hat{\beta}_k) + \sum_{k'=0, k' < k}^{k-1} (x_{ki}x_{k'j} + x_{kj}x_{k'i}) \text{C}\hat{\text{o}}\text{v}(\hat{\beta}_k, \hat{\beta}_{k'}) \right]$$

where $\text{Var}(\cdot)$ indicates the variance of the enclosed estimator, and $\text{Cov}(\cdot, \cdot)$ indicates the covariance of the 2 enclosed estimators. Thus,

$$\text{V}\hat{\text{a}}\text{r}(\hat{N}_h) = \sum_{i=1}^{U_h} \text{V}\hat{\text{a}}\text{r}(\hat{\psi}_i) + 2 \sum_{j=1, j < i}^{i-1} \text{C}\hat{\text{o}}\text{v}(\hat{\psi}_i, \hat{\psi}_j).$$

The covariance of pairs of $\hat{\psi}_i$ estimates, when they occur in strata h and h' ($h \neq h'$), was also computed with the above covariance estimator formula, but indicator variables were used to adjust for different intercepts between the 2 strata. The covariance between pairs of $\hat{\psi}_i$ estimates, when they occur in strata h and h' ($h \neq h'$), was needed to compute the covariance of the $\hat{N}_h = \sum_{i=1}^{U_h} \hat{\psi}_i$ between the 6 or 10 strata. For GUPD strata where the Division of Wildlife

Range covariate was not available, the x_{i1} or x_{1j} covariate value was taken as zero, and the formula reduces properly to the correct covariance. These formulae are different than those presented in Bowden et al. (2003) because they used a covariate to predict an estimated population size using a ratio estimator with correlated estimates, whereas our covariates are used to estimate directly the correlated estimates of occupancy rate.

Miscellaneous

Equipment: Equipment needed to conduct surveys may include all of the following: clipboards, waterproof pens, topographic maps, compasses, GPS units, battery chargers and rechargeable batteries, 10-power binoculars, backpacks, high lift jacks, tow chains, shovels, jumper cables, quadrat corner stakes, fluorescent red paint for corner stakes, hammers, thermometers, appropriate windspeed and temperature meters (i.e., Speedtech Instruments, Great Falls, Virginia), phone cards, and first aid kits.

Establishing Ownership of Quadrats: Plot ownership can be established by contacting County Assessor web sites and offices, reviewing plat books, and by contacting adjacent landowners. Contact information for lessees of State Land Board lands can be obtained from the State Land Board. Data sheets need to contain the plot number, owners name, address, and telephone number. The observer should record each phone call made to the landowner and special instructions such as need to notify a lessee shortly before visiting the land, access thru locked

gates, and if the owner desires a copy of the final report. If information on species other than prairie dogs is desired, landowners should be asked for permission to collect that data.

Informing Cooperators: Inform anyone who may be affected by surveys including Extension Agents, County Sheriffs, Bureau of Land Management, U.S. Forest Service, Division of Wildlife, Division of Parks and Outdoor Recreation, National Park Service, National Wildlife Refuges, State Land Board, The Nature Conservancy, Native American tribes, Natural Resources Conservation Service, and USDA/APHIS Wildlife Services.

Liability Issues: Some private landowners may be concerned about their liability for observers while they are on the landowner's property. In Colorado, our legal advisors believe that a landowner's liability to persons on their land would be covered under provisions of Section 13-21-115 of the Colorado Revised Statutes. Observers should be considered a "licensee" on private property. A landowner can only be found liable to a licensee if he/she fails in his/her duty owed to that other person as that duty is described in the statute. The statute limits the landowner's risk of liability, and should provide adequate protection to a landowner under normal circumstances.

GUNNISON PRAIRIE DOG OCCUPANCY SURVEY DATA FORM

NAME _____ DATE _____ PLOT NUMBER _____
STATE _____ STRATUM _____

UTM LOCATION OF THE SOUTHWEST CORNER OF PLOT NAD _____ Zone _____
Easting: _____ Northing: _____
ELEVATION OF PLOT USING GPS UNIT: _____

VISIT: First _____ Second _____

TIME START OBSERVATION _____
TIME END OBSERVATION _____
TOTAL TIME ON PLOT _____

PRAIRIE DOGS PRESENT: Yes No

MID-SURVEY:

TEMP: _____ % CLOUD COVER: _____ PRECIP: _____ WIND SPEED: _____

BURROWING OWLS ON PLOT: YES NO
NUMBER OBSERVED: ADULT _____ JUVENILE _____
NEST LOCATED YES NO

FERRUGINOUS HAWKS ON PLOT: YES NO
NUMBER OBSERVED: ADULT _____ JUVENILE _____
NEST LOCATED YES NO

KIT FOX ON PLOT: YES NO
NUMBER OBSERVED: ADULT _____ JUVENILE _____
DEN LOCATED YES NO

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Appendix C. Final Prairie Memorandum of Understanding

MEMORANDUM OF UNDERSTANDING
FOR
CONSERVATION AND MANAGEMENT OF SPECIES OF CONSERVATION CONCERN
ASSOCIATED WITH PRAIRIE ECOSYSTEMS

I. Purpose

The purpose of this Memorandum of Understanding (MOU) is to provide, under auspices of the Western Association of Fish and Wildlife Agencies (WAFWA), for interagency cooperation in conservation and management of species associated with prairie ecosystems of the Western Great Plains (i.e. parts of Arizona, Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, Wyoming, and Utah). The primary focus is on federally-listed species, state-listed species, and species of conservation concern. The participating agencies agree that cooperation is necessary to collect and analyze data on these species and their habitats, and to plan and implement actions necessary to establish and/or maintain viable populations of each species that are sufficient to preclude present or future endangerment, within the constraints of approved budgets.

Parties to this MOU are collectively referred to herein as Signatories.

II. Background

The Signatories have been involved in a variety of long-standing and recently initiated efforts to conserve and manage wildlife and habitats in the Western Great Plains. Many of these efforts have been conducted with a single species approach. Despite significant successes to date, the Signatories believe it is in their best long-term interest to move toward a landscape level approach that enables better planning and coordination, efficiency in time and scale of accomplishment, and greater cost effectiveness. The Signatories recognize that such a transition will take time, require adaptive management to respond to emerging needs and priorities, and present unique challenges in terms of process management, shared decision-making, and increased emphasis on community based conservation. They also recognize that as they move toward a landscape level or ecosystem focused, they must ensure that their commitment to conservation and management of individual species cannot be diminished such that imperilment occurs. Given these considerations, in 2004 WAFWA directed its Habitat and Nongame and Endangered Species committees to use renewal of an MOU for black-tailed prairie dog conservation as a vehicle for beginning the transition toward an ecosystem approach (i.e. prairie) in the Western Great Plains. WAFWA also directed the two committees to ensure that the prairie effort is fully coordinated with, and complementary to, a companion effort to conserve sagebrush

and sage-steppe communities (and associated species of wildlife) in the Great Basin, because the two biomes share many important species.

III. Objectives

The Signatories agree to accomplish the following conservation objectives:

1. Recognize that because the white-tailed prairie dog (WTPD) and Gunnison's prairie dog (GPD) inhabit sage-steppe and prairie scrub ecosystems rather than grasslands, they will fall under the purview of the WAFWA Sagebrush MOU when a new one is developed in 2007.
2. Develop a WTPD and GPD conservation strategy by January 31, 2006 to complement WAFWA's existing black-tailed prairie dog conservation strategy.
3. Develop state-specific prairie dog management plans, or integrate prairie dog management components into other state-specific and/or regional management plans, as appropriate, by December 31, 2007.
4. Develop a cohesive, comprehensive, WAFWA prairie conservation strategy by June 30, 2010 that integrates pertinent components of companion efforts for the WTPD, GPD, BTPD, black-footed ferret, swift and kit foxes, lesser prairie chicken, mountain plover, burrowing owl, ferruginous hawk, Swainson's hawk, loggerhead shrike, and, as appropriate and feasible, other shrub and grassland species in the Western Great Plains.
5. Coordinate with, establish, or otherwise convene various conservation teams, work groups, etc. as necessary to implement this MOU.
6. Cooperate to maintain and enhance, to the extent practicable, the populations and habitats of the species addressed pursuant to this MOU.
7. Coordinate with, as necessary and appropriate, companion conservation efforts in the United States, Canada, and Mexico.
8. Enhance awareness of the Signatories and local communities, industries, nongovernmental organizations, and private individuals regarding this conservation effort, and encourage and enhance their participation in partnerships to accomplish mutually agreeable conservation objectives.
9. Remain aware of, and inform WAFWA on, any legal, regulatory, or policy action associated with the species addressed pursuant to this MOU.

IV. Actions

1. WAFWA will identify a State Director to serve as Sponsor for this MOU.
2. The State Sponsor or their designee will:
 - a. Approve additional Signatories and modifications to this MOU;
 - b. Collaborate with IAFWA in contracting an Interstate Coordinator for this MOU;and

- c. Provide appropriate guidance to the Interstate Coordinator for managing this MOU, including (i) ensuring timely, effective coordination with the companion WAFWA conservation effort for sagebrush and sage-steppe habitats and the species therein; and (ii) integrating this conservation effort into WAFWA's support for development of a Western Shrubland Science and Management Information Consortium.
3. The Interstate Coordinator will serve as Chair for WAFWA's Prairie Dog Conservation Team and liaison to WAFWA's sagebrush and sage-steppe conservation program.
4. The Interstate Coordinator will facilitate the Signatories' efforts to identify and implement the most appropriate way(s) to collect data (e.g. range-wide survey and monitoring recommendations) for the species addressed pursuant to this MOU.
5. The Interstate Coordinator will assist WAFWA in integrating WTPD and GPD strategies into its sagebrush and sage-steppe conservation effort.
6. The Interstate Coordinator will facilitate Signatory cooperation in developing major media releases and media projects, as well as website support and other public outreach efforts, pursuant to this MOU.
7. The Interstate Coordinator will provide quarterly reports to WAFWA and IAFWA in April, July, and October, an Annual Report to WAFWA and IAFWA in February of each year, progress reports to WAFWA's Habitat Committee at annual WAFWA Summer Conferences and Mid-Winter Business Meetings, and an annual report to the Prairie Dog Conservation Team.
8. The Interstate Coordinator will provide appropriate grant progress reports to the National Fish and Wildlife Foundation in May 2006 (Phase 2 Report).
9. The Signatories will assist the Interstate Coordinator as necessary to ensure timely, effective, and well coordinated activities and completion of products and services pursuant to this MOU.
10. The Signatories will cooperate to maintain, and enhance to the extent practicable, viable populations and habitats of the species addressed pursuant to this MOU.
11. The Signatories will assist the Interstate Coordinator in ensuring local governments, communities, private citizens, and other interested and affected parties are informed on the status of this conservation effort, including ways that might provide local economic benefits.
12. The Signatories will recognize and respect the separate authorities of each signatory agency and the interests of other affected or interested parties.
13. The Signatories will cooperate in providing financial support for the Interstate Coordinator for this MOU, with a total annual budget of: YR1 \$112,000; YR2 \$112,000; YR3 \$116,000; YR4 \$118,000; and YR5 \$123,000 (the intent is for 50% of the stated annual amounts to be contributed by State Wildlife Agencies and 50% by Federal Agencies).
14. The Signatories will provide facilities, equipment, logistical support, authorizations, and permits as necessary and available to implement this MOU.

V. Authorities

This MOU is among various WAFWA States and the Bureau of Indian Affairs, Bureau of Land Management, Department of Defense, National Park Service, Natural Resources Conservation Service, U.S.D.A. APHIS Wildlife Service, U.S.D.A Forest Service, U.S. Fish and Wildlife Service, and U.S. Geological Survey, under provisions of the following Federal laws:

Federal Land and Policy Management Act of 1976 (43 U.S.C. 1701 et seq.)
Fish and Wildlife Act of 1956 (16 U.S.C. 742 et seq.)
Fish and Wildlife Coordination Act (16 U.S.C. 661-667)
Multiple-Use Sustained-Yield Act [of 1960] (16 U.S.C. 528-531)
Forest and Rangeland Renewable Resources Research Act of 1978 (16 U.S.C. 1641-48)
National Forest Management Act of 1976 (16 U.S.C. 1600 et seq.)
Endangered Species Act of 1973 (16 U.S.C. 1531 et seq.)
National Wildlife Refuge Administration Act of 1966, as amended by the National Wildlife
Refuge System Improvement Act of 1997 (16 U.S.C 668dd et seq.)

VI. Terms and Conditions

It is mutually agreed and understood by and between the Signatories that:

1. This MOU is neither a fiscal nor a funds obligation document. Nothing in this agreement may be construed to obligate Federal Agencies or the United States to any current or future expenditure of resources in advance of the availability of appropriations from Congress. Any endeavor involving reimbursement or contribution of funds between the Signatories to this MOU will be handled in accordance with applicable regulations, and procedures, including those for federal government procurement and printing. Such endeavors will be outlined in separate agreements that shall be made in writing by representatives of the Signatories and shall be independently authorized in accordance with appropriate statutory authority. This MOU does not provide such authority.
2. This MOU in no way restricts the Signatories from participating in similar activities with other public or private agencies, organizations, and individuals.
3. This MOU is executed as of the last date shown below and expires five years from the execution date, at which time it will be subject to review, renewal, or expiration.
4. Modifications within the scope of this MOU shall be made by issuance of a mutually executed modification prior to any changes being performed.
5. Any party to this MOU may withdraw with a 60-day written notice to the State Sponsor.
6. Any press releases with reference to this MOU, the Signatories, or the relationship established between the Signatories of this MOU, shall be reviewed by the Interstate Coordinator and State Sponsor prior to release.

7. In any advertising done by any of the Signatories, this MOU shall not be referred to in a manner that states or implies that any Signatory approves of or endorses unrelated activities of any other.
8. During the performance of this MOU, the Signatories agree to abide by the terms of Executive Order 11246 on nondiscrimination and will not discriminate against any person because of race, age, color, religion, gender, national origin, or disability.
9. No member of or delegate to Congress, or resident Commissioner, shall be admitted to any share or part of this agreement, or to any benefit that may arise from, but these provisions shall not be construed to extend to this agreement if made with a corporation for its general benefits.
10. The Signatories agree to implement the provisions of this MOU to the extent personnel and budgets allow. In addition, nothing in the MOU is intended to supersede any laws, regulations, or directives by which the Signatories must legally abide.

VII. Approval

In witness thereof, the Signatories hereto have executed this Memorandum of Understanding as of the last written date below.

Approved _____ Date _____
Arizona Game and Fish Department

Approved _____ Date _____
Colorado Division of Wildlife

Approved _____ Date _____
Kansas Department of Wildlife and Parks

Approved _____ Date _____
Montana Fish, Wildlife, and Parks

Approved _____ Date _____
Nebraska Game and Parks Commission

Approved _____ Date _____
New Mexico Department of Game and Fish

Approved _____
North Dakota Game and Fish Department

Date _____

Approved _____
Oklahoma Department of Wildlife Conservation

Date _____

Approved _____
South Dakota Game, Fish, and Parks

Date _____

Approved _____
Texas Parks and Wildlife Department

Date _____

Approved _____
Utah Division of Wildlife Resources

Date _____

Approved _____
Wyoming Game and Fish Department

Date _____

Approved _____
Bureau of Indian Affairs

Date _____

Approved _____
Bureau of Land Management

Date _____

Approved _____
Department of Defense

Date _____

Approved _____
National Park Service

Date _____

Approved _____
Natural Resources Conservation Service

Date _____

Approved _____
USDA APHIS Wildlife Services

Date _____

Approved _____
USDA Forest Service

Date _____

Approved _____
US Fish and Wildlife Service

Date _____

Appendix D. Plague protocol.

Prairie Dog Sylvatic Plague Monitoring Protocol

Since its documented appearance in wild rodents on the Pacific Coast of North America in the early 1900s, sylvatic plague has spread eastward to approximately the 103rd Meridian, affecting sciurid and cricetid rodents, insectivores, lagomorphs, carnivores, and humans (bubonic plague) (Barnes 1982; Cully 1993). Prairie dog species are extremely susceptible to this typically flea-borne disease and may serve as “amplifying hosts” (Barnes 1993).

Plague epizootics may originate from focal areas, with possible maintenance in non-focal areas between epizootics. During epizootics, plague can spread over great distances and in the process affect humans, most often during and shortly following epizootics (Cully 1993). Several wildlife species are considered enzootic or maintenance species for sylvatic plague, meaning individuals have some or considerable resistance to the disease. Examples include the California vole (*Microtus californicus*) in San Mateo County California, kangaroo rats (*Dipodomys* spp.), deer mice (*Peromyscus maniculatus*), and northern grasshopper mice (*Onychomys leucogaster*) (Cully 1993).

In the past, plague has been monitored for the protection of human health and conservation of prairie dog populations for ecosystem values, particularly protection of reintroduced populations of black-footed ferrets. As part of a range-wide commitment to prairie dog management, the Interstate Prairie Dog Conservation Team is developing this plague protocol to monitor and react to the threat of sylvatic plague on a range-wide basis.

Application of Deltadust Insecticide, a prophylactic treatment for flea control in burrows, is sometimes used prior to prairie dog relocation into plague-affected colonies (Dave Seery, pers. comm.) This technique may have limited applicability for flea control in other situations and is the only active treatment method currently available.

Sylvatic plague surveillance methods are summarized below.

Technique	Description
"Windshield surveys"	General observations of prairie dog towns to detect die-offs, with follow-up evaluations needed to confirm cause and status. Coordination with health professionals, field personnel, and private landowners will be important to achieve a valid sample of colonies statewide
Collection and analysis of dead prairie dogs	Prairie dogs often die in burrows, but a small percentage of those exposed to plague die above-ground and can be picked up if colonies are regularly surveyed for dead and dying prairie dogs
Collection and analysis of fleas from prairie dog burrows	This technique has had widespread use as a surveillance technique for human health concerns. It is a part of the Shirley Basin/Medicine Bow black-footed ferret plague contingency plan in Wyoming (Luce and Oakleaf 1994). Young et al. reported on using this technique on Fort Belknap Agency, Montana, and the Pueblo Chemical Depot in central Colorado
Collection of blood samples from members of Order Carnivora, especially coyotes and badgers	<p>Although such species as badgers and coyotes can become infected with plague, their primary role in the disease cycle is the transport of plague-infected fleas (Poland and Barnes 1979 cited in Gage et al. 1994). Nobuto blood-sampling papers have been used extensively, since the technique does not require access to refrigerators and requires only 0.2 ml of blood (Wolff and Hudson 1974, Gage et al. 1994).</p> <p>This technique has recently been used in association with black-footed ferret reintroduction, either via collection of blood samples from live animals, dead animals collected for this purpose, or animals killed during animal damage control activities (Anderson et al. no date, Williams et al. 1998, Matchett 2001). In addition, black-footed ferrets captured for removal of radio collars, for implantation of transponder chips, or for canine distemper vaccination can be bled for disease analysis samples.</p> <p>This technique can easily be incorporated into blood collection for other purposes, such as genetic analyses (NPWRC 1999).</p>
Collection of blood samples from domestic dogs	Barnes (1982) reported using domestic dogs as sentinels for exhibiting antibodies to plague. This technique has been effective on Native American reservations in the Southwest to detect seroconversion before plague was observed in rodents or humans.
Collection of blood from potentially resistant small mammals	Certain rodent species appear to be resistant to plague and may serve as maintenance or enzootic hosts that maintain plague between epizootics (Cully 1993, Gage et al. 1994).

Technique	Description
	The Wyoming Game and Fish Department has monitored small mammals for plague seroconversion in Shirley Basin, Wyoming (Luce et al. 1994, 1996, 1997). Trapping efforts focused on deer mice and grasshopper mice, with the assumption that active plague would be detectable by antibodies produced during the short life span of these rodents. These investigations detected a relationship between seroprevalence of plague in deer and grasshopper mice and status of white-tailed prairie dog populations in Shirley Basin.

ACTIONS:

1. State wildlife agencies will initiate a public information program to inform landowners, hunters, and other members of the public concerning the need to notify the agency of die-offs of prairie dogs or ground squirrels.
2. State wildlife agency prairie dog coordinators, in cooperation with state public health officials, will take the lead to inform state Department of Agriculture, USDA-Wildlife Services, NRCS, veterinarians, and local government personnel that deal with animal control, or have regular contact with landowners and the public, of the need for reporting die-offs.
3. State wildlife agency prairie dog coordinators, in cooperation with state public health officials, will take the lead in providing information and training for state Department of Agriculture, USDA-Wildlife Services, NRCS, veterinarians, and local government personnel that deal with animal control, on protocols for collection of dead prairie dogs and ground squirrels, packaging and record keeping.

The CDC and Wyoming State Veterinary Laboratory (WSVL) both have extensive experience conducting disease surveillance in wild mammals. CDC does not charge for diagnostic services, but has limited laboratory capacity. The eleven black-tailed prairie dog states will use CDC, individual state diagnostic labs, or WSVL diagnostic services for examination of prairie dog and ground squirrel carcasses for disease detection. Although other laboratories can provide a similar service as the WSVL, there would be a significant advantage in having all of the diagnostic examination done at a lab that is familiar with the procedures, will produce consistent results, and will report them state by state for the eleven states as the WSVL has done for black-footed ferret reintroduction sites for several years. In addition to testing for plague, specimens will be tested for tularemia, pasteurellosis, undetected poisoning, drowning, and predator kill.

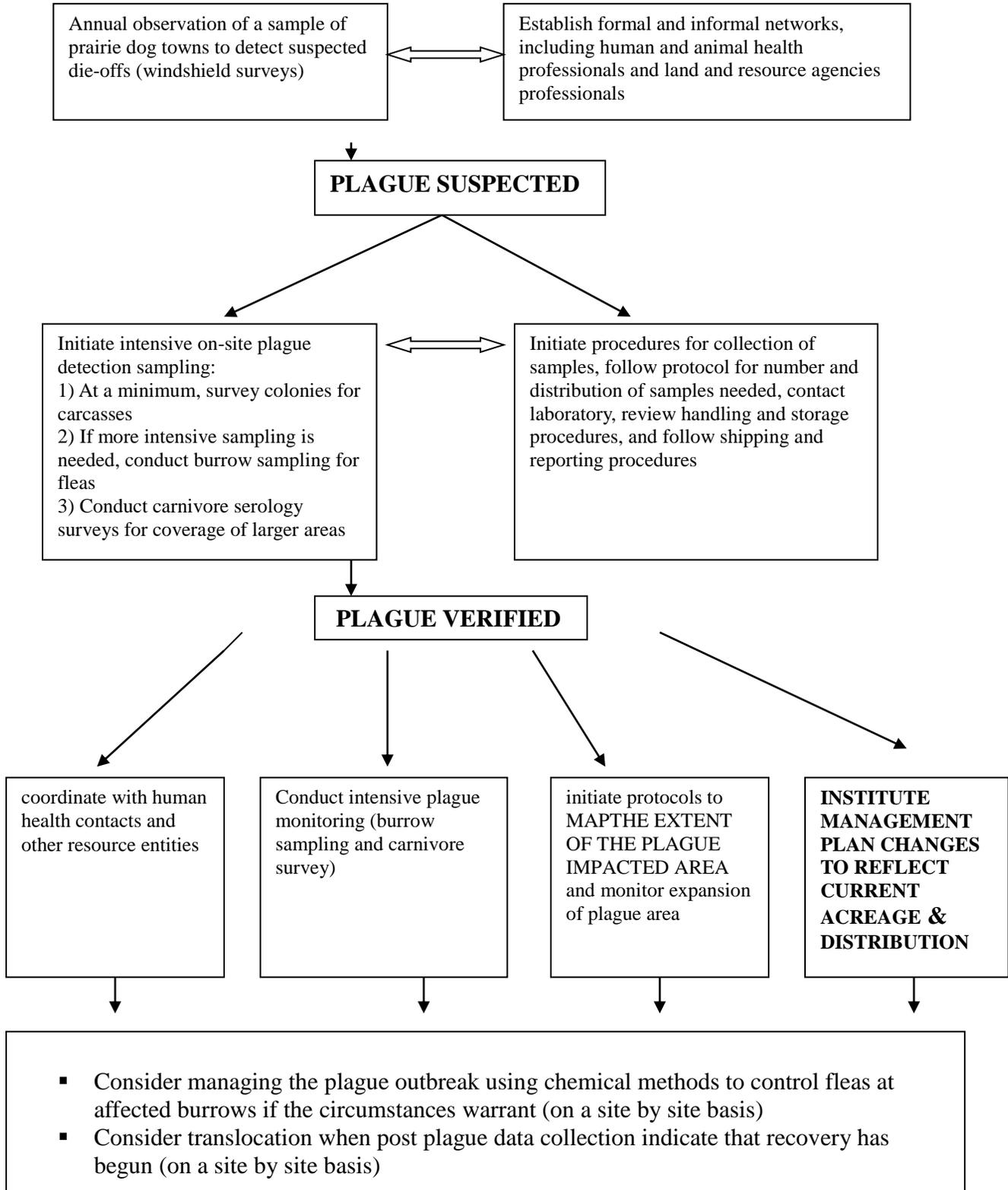
4. State prairie dog coordinators will develop windshield survey routes throughout the prairie dog range to be conducted annually by wildlife agency or other personnel in each county, or smaller unit where prairie dogs occur, during March and April. Windshield surveys will

follow the CDC protocol (Enscore pers. comm.)(Appendix 1). Significant decline in any colony or complex should be immediately reported to the state prairie dog coordinator.

In the event of a suspected die-off (if a windshield survey route reports a significant loss of prairie dogs or ground squirrels), the state will implement the plague contingency plan immediately (Appendix 2).

- A. Make local inquiries to determine whether or not the colony was poisoned, or whether mortalities were due to heavy shooting
 - B. If neither shooting nor poisoning occurred, the colony or complex should be searched for prairie dog and ground squirrel carcasses as soon as possible after discovery of the population decline. Carcasses should be handled in the field according to protocol (Appendix 2)
 - C. In the event that carcasses cannot be found, and the disappearance of prairie dogs is verified as recent, burrow swabbing should be conducted to collect fleas according to CDC protocol (Appendix 3)
6. If plague is verified, the prairie dog coordinator, in cooperation with state public health officials and CDC, should immediately notify, and make plague contingency recommendations to, the following: landowners and wildlife agency personnel in the affected area, state Department of Agriculture, USDA-Wildlife Services, NRCS, veterinarians, and local government personnel that deal with animal control, and the general public through local media sources.
 7. Post-plague monitoring of prairie dog colonies should be conducted annually in March or April to document the rate of re-colonization and verify occupied acreage. Initial monitoring, which will take place from one to several years, should consist of windshield surveys. When visual surveys indicate prairie dog colonies are recovering, a quantitative survey method should be initiated. The recommended method, due to widespread use, particularly on black-footed ferret reintroduction sites, is transecting using the Biggins method (Biggins et. al. 1993) that equates active and inactive burrow densities to population density.
 8. The prairie dog coordinator and the prairie dog working group should evaluate the extent of the impact of the epizootic as it affects the acreage and distribution objectives in the management plan. The group should determine whether or not there is a need to modify prairie dog management in the plague area, and potentially elsewhere in the state, if occupied acreage is below the objectives in the state management plan.

Plague Monitoring Framework



Appendix 1

Centers for Disease Control Procedure for Visual Evaluation of Prairie Dog Colonies for Plague in the Southwestern United States

Citation: Ensore, R. personal communication. Undated. Centers for Disease Control and Prevention, NCID, Division of Vector Borne Infectious Diseases, Plague Section, Fort Collins, Colorado. 3pp.

HEALTHY COLONY

OBSERVATION: The vast majority of burrows show signs of recent use, unless it has rained within the past 24 hours – in which case the colony should be reexamined following a period of at least 24 hours without precipitation. Active prairie dogs are observed during periods of acceptable weather conditions. Only a relatively few (<10%) burrow openings appear inactive (lack of disturbed dirt, presence of cobwebs or wind-blown vegetation over the entrance). An occasional carcass or dried bones may be present as a result of non-plague death or predation.

EVALUATION: Unless recently (days) introduced, plague is not likely to be present. Fleas are not likely to test positive.

SAMPLE RECOMMENDATIONS: No samples recommended.

DEAD COLONY

OBSERVATION: The colony appears completely inactive. Burrows show no signs of recent use (re-examine if it has rained within 24 hours). An occasional desiccated carcass and bones may be present, and have likely been scavenged.

EVALUATION: 1) Make inquiries to determine if the colony was poisoned. This is especially likely if it appears that dirt was shoveled into the burrows. If there is no evidence of poisoning and the food supply appears ample: 2) it is likely that plague or some other zoonotic disease killed the colony. An experienced observer can usually make an estimate (recently, 1 season, or 2 seasons) on how long the colony has been inactive by considering the soil type and degree of burrow degeneration.

SAMPLE RECOMMENDATIONS: Sample only if there is no evidence of poisoning. A recent (same season) die-off might produce many fleas through burrow swabbing. Older die-offs will likely produce few or no fleas. Typically, many burrows (dozens or even hundreds) may be swabbed with only a few producing fleas. If burrowing owls are using the inactive burrows,

small black stick-tight fleas may be present in large numbers (in contrast to the larger, reddish-brown prairie dog fleas). Fresh or desiccated prairie dog carcasses may also be collected for analysis.

SCATTER PATTERN:

OBSERVATION: Inactive burrows constitute an unusually high (typically 20-90%) percentage of the total burrows. Active burrows however are clearly evident and active prairie dogs are observed during periods of acceptable weather. Active and inactive burrows are scattered amongst each other in no particular pattern (see below), keeping in mind that family units may have multiple burrow openings and hence an inactive unit may produce a small cluster of 2-5 inactive burrow openings. An occasional carcass (fresh or desiccated) and bones may be present.

EVALUATION: Several scenarios could account for these observations – and more than one scenario may be in play at the same place and time. Presented in order of likelihood: 1) Make inquiries to determine if the colony was poisoned. This is especially likely if it appears that dirt was shoveled into the burrows. This scatter pattern could be produced if the application of poison was scattered and not comprehensive, 2) If there is no evidence of poisoning, assess the available food supply. Such a pattern of death could also be attributable to a population crash as a result of lost carrying capacity of the site or over-population, 3) If there is no evidence of poisoning or population crash, hunting by humans or excessive predation by carnivores or birds of prey are highly likely. Human hunting usually produces physical evidence such as footprints, tire tracks and spent ammunition shells. Depending upon the local culture, human hunters may collect their prey (many Native American groups regard prairie dogs as a delicacy) or leave it for scavengers. Experienced observers can often spot carnivore tracks and recognize hunting and attack patterns in these tracks near burrow entrances, 4) Finally, a zoonotic disease could be responsible, but given this mortality pattern, a disease with a lower mortality rate than plague is more likely.

SAMPLE RECOMMENDATIONS: If there is no evidence of poisoning, population crash, or excessive human hunting: collect fleas by swabbing burrows – especially inactive burrows – and collect fresh or desiccated prairie dog carcasses if available.

DEAD ZONE

OBSERVATION: Within an otherwise healthy appearing colony, there is a zone of inactive burrows. This zone may encompass a relatively small or large proportion of the colony, and may be located anywhere in the colony. Eventually it spreads to encompass a section of the colony and appears to be spreading, along a discernable line of demarcation over the remaining section of the colony. Experienced observers can often clearly distinguish and mark (flagging tape) this demarcation line between active and inactive regions. Marking allows for periodic re-examination to assess the rate of spread and facilitates sampling. Fresh or desiccated carcasses

may be present. Near the demarcation line, recently inactive burrows may reveal the odor of decaying carcasses and flies may be common at burrow entrances.

EVALUATION: 1) There is a high probability that plague is active in such a colony. Although other zoonotic diseases are possible, plague is most likely, 2) Depending upon the location of the dead zone with respect to other human activity (homes, barns, etc.) poisoning is also a possibility and should be investigated.

SAMPLE RECOMMENDATIONS: Collect fleas by swabbing burrows immediately along both sides of the demarcation line, concentrating a majority of your efforts immediately along (within 10meters) the inactive (dead) side of the line. Fleas are likely to be numerous. You may wish to apply extra insect repellent but be extremely cautious not to directly or indirectly get repellent on your burrow swab! (If this happens: discard it, wash your hands, and start with a new one). If others in a group are getting fleas and you are not, and you are swabbing essentially the same area, you likely have repellent on your swab. Collect any available rodent carcasses (fresh or desiccated, prairie dog or other rodent) for testing.

Additional Notes: Please include GPS coordinates for all samples. One set of coordinates per colony is acceptable. Specify the type of inactivity pattern noted for each sampled colony: dead colony, scatter pattern, dead zone. Analysis of samples from "dead zone colonies" will receive laboratory priority.

The above activity patterns are typical for the warm months. Visual examination during winter months is more difficult due to decreased daily activity among even healthy animals.

Appendix 2

Field Procedures for Collecting and Handling Carcasses as Diagnostic Specimens

1. Search prairie dog colonies systematically using walking or 4-wheeler transects spaced at about 50 meters.
2. When a carcass is discovered, ascertain if possible, whether or not the animal was shot. If mortality by shooting is confirmed there is no need to collect the specimen.
3. Before you collect a carcass, prepare a tag with the following information: species, date, location (both legal description and UTM is recommended), name of collector, agency or affiliation of collector, telephone number and address of collector, brief description of circumstances for collection.
4. When collecting a carcass, the collector should wear leather or latex gloves, and a long sleeved shirt or jacket that is tight at the wrist, to ward off fleas.
5. Invert a one-gallon plastic ziplock freezer bag over your hand, grasp the carcass in your hand, quickly fold the bag over the carcass, roll the bag on the ground, away from your body, to expel the air, and seal the ziplock.
6. Immediately place in a second ziplock bag, put in the tag, roll and seal the second bag.
7. As soon as possible after collection, freeze the specimen.
8. Sample Size:
 - A) If specimens are from a single sample area (one prairie dog colony or area) collect as many specimens as is practical up to 15, but initially ship only the freshest five specimens to the diagnostic lab.
 - B) Freeze the additional specimens that were collected, up to ten, and save for further testing needs, depending upon the results from the testing of the first five specimens. Keep the samples until notified by the WSVL or other lab that results were obtained from the first five samples and that the additional specimens will not be needed.
9. Ship the frozen specimen to WSVL, CDC, or designated lab.

(DO NOT USE UPS). U.S. Postal System or FEDEX can ship carcasses that are sealed in plastic bags and a cardboard box. Their regulations require:

- A) Carcasses must be individually labeled and bagged in watertight bags (minimum triple bag in ziplocks)
 - B) Placement of absorbent packing material around the carcass (crumpled newspaper, etc.)
 - C) Use of approved laboratory shippers or hard-sided containers, adequately taped closed
 - D) Marking of the container with "Biomedical Material" label (for U.S. Postal Service) or shipped as hazardous material by Federal Express (requires a special form and should be labeled as Diagnostic Biomedical Material on the form. Labels and forms may be obtained from the U.S. Postal Service or Federal express.
 - E) Carcasses should be frozen or packed with frozen ice packs (no wet ice).
10. Cost: WSVL cost for testing for plague, tularemia, pasteurellosis, undetected poisoning, and predator kill is a maximum of \$60.00 per specimen. CDC testing is free but the Ft Collins laboratory has limited capacity and can handle no more than 50 specimens per year.
11. Contact before shipping:

Wyoming State Veterinary Lab
1174 Snowy Range Road
Laramie, WY 82070
307-742-6638

or

(Shipment by U.S. Postal System)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
P.O.Box 2087
Ft. Collins, CO 80522

(Shipment by FEDEX)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
Rampart Road (CSU Foothills Campus)
Fort Collins, CO 80521

Appendix 3

Centers for Disease Control Procedure for Flagging (Swabbing) Rodent Burrows

- Citation: Gage, K. Personnel Communication. Undated. Centers for Disease Control, Ft. Collins, CO. 3pp.
- Leon Carter: 970-221-6444 (Biologist, Diagnostic and Reference Section - Responsible for handling specimens and doing much of the plague-associated laboratory work at CDC.)
- Ken Gage: 970-221-6450 (Plague Section Chief - Responsible for CDC's plague surveillance and control program. Trained as medical entomologist/zoologist)
- Rusty Ensore: 970-221-6452 (Environmental Health Specialist IV, Plague Section – Registered Sanitarian)
- John Montenieri: 970-221-6457 (Biological Technician, Plague Section - GIS specialist)

Some important flea vectors of plague infest rodent species that live in burrows. Although these fleas usually can be found in abundance on live hosts, they also can be collected by a procedure known as burrow flagging or burrow swabbing.

This procedure requires:

- 1) Burrow swabbing device consisting of a flexible cable, wire, or strong rubber hose with spring-loaded clip attached to the end. We prefer a steel plumber's "snake" that has an alligator clip screwed on the end as a means of attaching the flag. A simple burrow swab can be made by attaching a flag to the end of a piece of wire (about the thickness of a coat hanger), but this primitive swab allows only the top 2 or 3 feet of a burrow to be swabbed and will miss some fleas. Despite the shortcomings of the latter technique, it can be useful when die-offs are encountered unexpectedly and more sophisticated means of swabbing fleas are not available.
- 2) Flags consisting of white flannel cloth squares (approx. 25 cm² or 10 in²). We prefer white flannel because it is easier to see the fleas on white cloth than on cloths of other colors. Flannel is better than most other cloths because of its deep nap, which increases the likelihood that fleas will continue to cling to the cloth flag after it is removed from the burrow.
- 3) Plastic bags (approx. 20-40 cm² or 8-15 inches)(Zip-loc type are best)
- 4) Insect repellent (DEET) to spray on clothes and exposed skin on arms, legs, etc. Although this is recommended for safety reasons, care must be taken not to apply repellents to hands

because the repellent is likely to transfer to the flagging material, thus preventing fleas from jumping onto the flag. Note: Clothing also can be treated with permethrin-containing sprays but these sprays should not be applied directly to the skin.

Procedure:

1. Attach a flag to the clip on the end of the burrow swab.
2. Force the flag as far as possible down the burrow. The fleas confuse the flag with their normal host and cling to it as it passes through the burrow.
3. Slowly withdraw the flag from the burrow after approximately 30 seconds.
4. Quickly place the flag in a plastic bag.
5. Seal the bag to prevent the fleas from escaping.
6. Keep track of the number of burrows swabbed so that a burrow index can be calculated.

Burrow index = no. fleas collected/no. burrows sampled - This value often increases dramatically during die-offs among prairie dogs, rock squirrels, California ground squirrels, or other ground squirrel species)

7. Place another flag on the swab and repeat steps 1-6 for each burrow.
8. Transport flags back to laboratory in the plastic bags. Keep the bags in a reasonably cool place to prevent desiccation of the flea samples (*Yersinia pestis* is very susceptible to death by desiccation) or death of the plague bacilli due to excessive heat (remember pick-up hoods can get very hot in direct sunlight! Fried samples will come back negative for plague every time!).
9. Place bags in freezer overnight to kill the fleas.
10. Place the flags and loose contents of the plastic bags in a white enamel pan. Fleas may be picked from the flags and bottom of the pan with forceps.
11. Place fleas in vials containing 2% saline and a very small amount of Tween-80 detergent (<0.0001% of solution). Remember the detergent is added to reduce surface tension and allow the fleas to sink to the bottom of the vial. Too much detergent will kill the plague bacteria and prevent successful isolation. Fleas can be submitted in 2% saline without Tween-80, but an effort should be made to submerge the fleas. If the fleas have been killed by freezing, this should not be a problem. Although not recommended for routine collecting,

some investigators occasionally remove live fleas directly from the flags and place them in vials of saline. Live fleas placed in saline containing the Tween-80 detergent will be unable to float on the surface of the liquid, thus ensuring that they will drown soon after being placed in the saline. Without the detergent, surface tension can become a problem because the numerous bristles and setae found on fleas enable them to remain afloat on the surface of saline. This can be a potential safety problem because floating fleas often survive shipment and arrive at the laboratory ready to jump onto lab personnel. Rapid freezing of the fleas obviously eliminates this problem, but adding Tween-80 to the saline also helps reduce the growth of fungi on flea samples. Dead fleas trapped in the surface tension at the air-saline interface rapidly become overgrown with fungi making identifications more difficult.

12. Vials containing 2% saline and fleas can be shipped to CDC for taxonomic identification and analysis of the fleas for *Yersinia pestis* infection. The fleas can be shipped at ambient temperature in the vials of 2% saline. For best results, ship the specimens as soon as possible because the fleas will start to decay soon after collection. Be sure and double wrap the vials in a leak-proof material and then place them in a crush-proof box or metal mailing tube for shipment to CDC.

13. CDC Address by U.S. Postal System
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
P.O.Box 2087
Ft. Collins, CO 80522

Shipment by FEDEX:
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
Rampart Road (CSU Foothills Campus)
Fort Collins, CO 80521

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