MANAGEMENT PLAN FOR THE BLACK-FOOTED FERRET IN ARIZONA

Arizona Game and Fish Department

Technical Report 301
Nongame and Endangered Wildlife Program
Terrestrial Wildlife Branch
Arizona Game and Fish Department
5000 West Carefree Highway
Phoenix, Arizona 85086

December 2016

Photo by George Andrejko

Recommended Citation

Arizona Game and Fish Department. 2016. Management Plan for the Black-footed Ferret in Arizona. Nongame and Endangered Wildlife Program Technical Report 301. Arizona Game and Fish Department, Phoenix, Arizona.

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Project Funding

Funding was provided by the Arizona Game and Fish Department's Heritage Fund, State Wildlife Grants, and Wildlife Restoration Funds.

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INTRODUCTION

Very few historical data exist for black-footed ferret (*Mustela nigripes*; hereafter BFF) populations in Arizona. While the U.S. Fish and Wildlife Service (USFWS) established a national captive breeding program, the Arizona Game and Fish Department (Department) began investigating the Arizona landscape for suitable reintroduction sites. The extirpation of the black-tailed prairie dog (*Cynomys ludovicianus*; hereafter BTPD) from southeastern Arizona in the 1960s focused the Department's efforts on northern Arizona and reestablishment within the Gunnison's prairie dog (*Cynomys gunnisoni;* hereafter GPD) range. Over the course of 10 years, the Department evaluated the habitat and prey populations necessary to establish a viable Arizona BFF population.

The Department initially focused on the Aubrey Valley, near Seligman, Arizona. A *Cooperative Reintroduction Plan for Black-footed Ferrets Aubrey Valley, Arizona* (Belitsky et al. 1994) guided the Department's early efforts of on-site captive breeding, conditioning, and releases. The Department re-evaluated these efforts in 2003 when reintroduction began to be successful (Van Pelt et al. 2003). Ten years after the first release, and with the apparent success of the Aubrey Valley reintroduction, the Department attempted to establish a second population within the Espee Allotment, Babbitt Ranches, near Williams, Arizona (King et al. 2007). However, the presence of sylvatic plague within the GPD population has negatively affected the success of this second reintroduction effort (AGFD unpublished data).

After their listing under the Endangered Species Act (ESA), and with increased knowledge of the species and their associated threats, the USFWS has twice revised the initial *Recovery Plan for the Black-footed Ferret* (USFWS 1978, 1988, 2013). The *Recovery Plan for the Black-footed Ferret* (USFWS 2013) updated recovery goals and actions, and included recommendations for each state to achieve in order to meet national recovery objectives. To meet the recommendation for Arizona, the Department has produced this statewide management plan.

LEGAL STATUS

Endangered Species Act

The USFWS listed the BFF as endangered under the Endangered Species Preservation Act in 1967 (32 Federal Register (48):4001), the Endangered Species Conservation Act in 1970 (P.L. 91-135, 83 Stat. 275), and under the ESA in 1974 (16 U.S.C. 1531-1544, 87 Stat. 884). Even with the enactment of these protections, the species declined until 1979 when it was believed to be extinct. However, in 1981 researchers discovered a small population in Meeteetse, Wyoming (USFWS 2013). This population soon began to decline due to disease (i.e. canine distemper and

sylvatic plague), which prompted the USFWS to capture the remaining BFF from 1985-1987 and place them in a captive breeding program to save the species from extinction (USFWS 2013).

Section 10(j) Nonessential Experimental Population in Arizona – In 1991, while evaluating potential BFF habitat, the Department initiated a statewide effort to determine the public's attitude toward BFF reintroduction. Through this process, the Department decided the designation of a nonessential experimental population (as prescribed in Section 10(j) of the ESA of 1973, as amended) would be necessary to achieve a viable BFF reintroduction project with landowner support in Arizona. This designation allows for flexibility in managing the reintroduced population by reducing the liability on individuals who may adversely impact BFF while conducting otherwise lawful activities. The Department proposed, and the USFWS completed, a final rule in March 1996 designating the Aubrey Valley Nonessential Experimental Population Area (AVEPA), in part, to evaluate and refine release techniques for the species (Belitsky et al. 1994, 61 Federal Register (55): 11320-11336).

Programmatic Safe Harbor Agreement
To encourage landowners to engage in the conservation of the BFF, and to offer assurances that no future restrictions will be imposed due to the presence of a reintroduced endangered species, in 2013 the USFWS established the *Black-Footed Ferret Programmatic Safe Harbor Agreement* (USFWS 2013). Willing landowners who voluntarily enroll in the Programmatic Safe Harbor Agreement will allow for the reintroduction of BFF on their properties, and may withdraw from the agreement without penalty. In 2016, two landowners in Arizona participated in the Programmatic Safe Harbor Agreement: the Babbitt Ranches LLC for the Espee Allotment, and Seibert Land Company LLC for the Double O Ranch.

Arizona Revised Statute Title 17
General provisions of Arizona Revised Statutes, Title 17 protects all of Arizona's native wildlife, including federally listed threatened and endangered species. The Department includes the BFF on the Species of Greatest Conservation Need Tier 1A (AGFD 2012). The list provides policy guidance on management priorities only, not legal or regulatory protection.

RECOVERY PLANS

USFWS 1978 Recovery Plan
The primary objective of the 1978 Recovery Plan (USFWS 1978) was to maintain at least one wild self-sustaining population of BFF in each state within its former range. The USFWS described mechanisms to reach the primary objective, but did not identify additional objectives due to the lack of knowledge on the species.

USFWS 1988 Recovery Plan
Objectives in the 1988 Recovery Plan (USFWS 1988) included the following:

1. Increase the captive breeding population of BFF to 200 breeding adults by 1991.
2. Establish a pre-breeding census population of 1,500 free-ranging breeding adults in ten or more populations with no fewer than 30 breeding adults in any population by 2014.
3. Encourage the widest possible distribution of reintroduced BFF populations.

USFWS 2013 Recovery Plan

The 2013 Recovery Plan states three objectives (USFWS 2013):

1. The continued efforts of captive breeding facilities to provide animals of suitable quality and quantity for release into the wild.
2. The conservation of prairie dog habitat adequate to sustain BFF in several populations distributed throughout their historical range.
3. The management of sylvatic plague to minimize impacts to BFF at reintroduction sites.

To reach these objectives, the Recovery Plan recommended each of the 12 states (Arizona, Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, Utah, and Wyoming) initiate or maintain one or a combination of the following types of reintroduction efforts within the species' range to accomplish national objectives:

- One or more large size BFF reintroduction sites with the potential for more than 100 adult breeding BFF,
- One or more medium size BFF reintroduction sites with the potential for 50–100 adult breeding BFF, and
- One or more small size BFF reintroduction sites with the potential for 30–50 adult breeding BFF.

In addition to these recommendations, the Recovery Plan outlined state-specific population targets that if accomplished by each state, would lead to the range-wide recovery of the species. Under these recommendations, Arizona recovery includes a minimum population of 74 adults occupying 17,000 acres of prairie dog habitat to downlist the species to threatened, and 148 adults occupying 34,000 acres of prairie dog habitat to delist. The USFWS requires maintenance of these population levels for a minimum of three years to achieve state-level recovery.

MANAGEMENT PLAN GOAL

The Department's intentions with this statewide management plan are to: 1) define a process for statewide BFF recovery through the establishment of multiple populations; 2) define a mechanism to measure success for each population; and 3) outline management strategies and regulatory changes to implement this plan. Arizona's BFF population in Aubrey Valley appears to be in a downward trend (2015), and the cause is unknown. This trend emphasizes the need for a plan that will outline how the Department will recognize, respond, and adapt to challenges to achieve statewide recovery and meet the recommendations in the Recovery Plan (USFWS 2013). Achieving statewide recovery with only one population, in only one area, increases vulnerability of extirpation. While the contiguous distribution of the species in inter-connected populations would be ideal, the Department will lessen the effects of any localized or stochastic event through the establishment of multiple BFF populations statewide.

While the Department assumes primary responsibility for the implementation of this state plan, ultimately the support of federal, state, local, Native American, private partners of the BFF Working Group, and members of the public will be the foundation for its success. Currently, those partners include U.S. Department of Agriculture Animal and Plant Health Inspection

Service (USDA-APHIS), Arizona State Lands Department (ASLD), Babbitt Ranches, Havasupai Tribe, Hopi Tribe, Hualapai Nation, Natural Resource Conservation Service, Navajo Nation, The Phoenix Zoo, the U.S. Forest Service Kaibab National Forest, National Park Service, USFWS, and private land managers. The Department will continue to cultivate opportunities to work with any individual or organization to achieve recovery.

This management plan is divided into four parts: 1) a brief history of BFFs and reintroduction efforts in Arizona; 2) a process for Arizona recovery and the mechanisms and locations to reach recovery; 3) an outline of conservation and management techniques and protocols the Department will implement statewide; and 4) the federal regulatory changes necessary to implement this plan with landowner support.

HISTORY OF BLACK-FOOTED FERRET IN ARIZONA

HISTORICAL OCCURRENCE

Prehistoric evidence of BFFs in the Southwest derives from a bone fragment discovered in Jimenez Cave, Chihuahua, Mexico (Messing 1986). Prior to European settlement, BFFs occurred within Arizona and into Mexico within the historic ranges of black-tailed and GPDs (Hillman and Clark 1980). At the turn of the 19th century, nationwide use of rodenticides and agricultural disking reduced prairie dog populations, resulting in a decline of BFF populations. In Arizona, BFF museum specimens were collected from three locations in Coconino County from 1929 to 1931 (Hoffmeister 1986), and in 1967, Animal Damage Control personnel reported seeing BFF sign while poisoning prairie dogs (Belitsky et al. 1994). The exact date of BFF extirpation in Arizona is unknown, although there were no verified reports in Arizona from 1967 to 1996, after which reintroduction efforts began in AVEPA.

REINTRODUCTION IN ARIZONA

Black-footed Ferret Reintroduction Plan

In 1994, the Department drafted *A Cooperative Reintroduction Plan for the Black-footed Ferrets Aubrey Valley, Arizona* with the purpose to "describe the management actions necessary to re-establish a naturally breeding, self-sustaining population of ferrets" with the goal to "re-establish at least one wild ferret population that maintains a total of at least 53 breeding-aged adults (Belitsky et al. 1994)." The objectives of the plan were to:

1. In compliance with the USFWS BFF Recovery Plan, manage one reintroduction site in Arizona with >30 breeding adults, and retain enough prairie dog habitat to support these BFF.
2. Cooperatively work with the ASLD and landowners in the management area to maintain at least 90 percent of the prairie dog acreage known in 1992 (17,735 acres).
3. Promote a working relationship among the Department, Navajo Natural Heritage Program, USFWS, The Phoenix Zoo, ASLD, and landowners.

4. Initiate BFF reintroduction into Aubrey Valley in 1994. If Aubrey Valley should fail as the priority experimental reintroduction site in 1994, use the site in the future when it meets the minimum criteria.
5. Reintroduce up to 50 BFF initially; annually reintroduce an adequate number to establish a population with >30 breeding adults by 1998.

In 2003, the Department produced a *Review of Black-footed Ferret Reintroduction in Arizona, 1996-2001* (Van Pelt and Winstead 2003). The document examined the Department's management practices and outlined recommendations for future reintroduction efforts. These recommendations were:

1. Replacement of a portion of the pre-conditioning pens with pens using a design to eliminate predation by raptors, and reduce maintenance costs (material and personnel time).
2. Monitor prairie dog populations within the release area using standardized annual surveys and use of the resulting data to determine sites suitable for future BFF releases.
3. Expend no less than 800 hours per year conducting spotlight surveys. If necessary, refine protocols (e.g. timing, length, route location) and test other forms of monitoring (track-plates).
4. Develop a reliable radio telemetry system to determine BFF dispersal, survival, and habitat use.
5. Continue evaluating spring releases of BFF.
6. For releases in autumn, request no less than 30 kits.

This management plan builds upon these previous recommendations and defines a mechanism to determine an expected number of BFF family groups for each population.

Aubrey Valley/Double O Ranch

In 1985, the Department began investigating the possibility of re-establishing BFF in Arizona (Belitsky et al. 1994, VanPelt 1995). After evaluating eight GPD complexes across northern Arizona, the Department selected the Aubrey Valley for the first reintroduction as it contained one of the highest concentrations of GPD in the state (Van Pelt 1995).

In September 1996, Aubrey Valley became the fifth BFF reintroduction site in the United States with the initial release of 35 BFF (BFFRIT 2011), and the first reintroduction site into a GPD population. From 1996-2000, 132 BFF were released within the Aubrey Valley, but no wild born kits were ever detected. In 2001, the Department modified release strategies to incorporate pen breeding and springtime releases to coincide with timing of GPD birth cycle (i.e. maximum food availability) to promote BFF reproduction. This effort was successful in producing the first wild-born kits in 2001. Through 2015, the Department released 418 BFFs into the Aubrey Valley.

The Double O Ranch, Seibert Land Company, is located southeast of the Aubrey Valley but within contiguous GPD habitat. In 2016, in anticipation of BFF population expansion to GPD-occupied habitat on the Double O Ranch, the Seibert Land Company enrolled in the Programmatic Safe Harbor Agreement and released six BFFs.

Espee Allotment, Babbitt Ranches

The Espee Allotment, Babbitt Ranches, was one of eight original GPD complexes investigated for BFF reintroduction by the Department, GPD-occupied acreage in 1996 was insufficient to support a BFF population. In the summer of 2007, the Department mapped GPD towns on the Babbitt Ranches to re-estimate GPD acreage and density, and as a result, began the process to establish a second BFF reintroduction site in Arizona on the Espee Allotment (King et al. 2007). Late in 2007, the Espee Allotment became the 14th BFF reintroduction site in the United States with the initial release of 44 BFF under the Department's ESA Section 10 permit. After 2013, Babbitt Ranches enrolled in the Programmatic Safe Harbor Agreement to release BFF. Through 2015, the Department has released 99 BFFs into the Espee Allotment. However, 2015 spotlighting documented no BFFs.

BLACK-FOOTED FERRET POPULATION NUMBERS AND MANAGEMENT AREAS

The BFF Recovery Plan recommends an Arizona population of 148 breeding adults on 34,000 GPD-occupied acres maintained for three consecutive years to meet delisting criteria (USFWS 2013). In an effort to reduce the effects of stochastic events on a single population in Arizona, the Department proposes to establish and manage multiple BFF populations across Arizona. At a minimum, and to reach Arizona's 148 BFF, the Department plans to establish and sustain three to five populations with no fewer than 30 breeding adults in any population to reach a minimum 148 animals. Some locations may sustain more than 30 breeding adults, and the Department may attempt to establish populations in other locations not described within this plan if opportunities arise. Regardless, additional animals and populations will ultimately contribute to meeting the minimum 148 breeding adults for Arizona.

MECHANISM TO DETERMINE EXPECTED NUMBER OF FAMILY GROUPS

Estimates of the minimum white-tailed prairie dog (*Cynomys leucurus*) occupied acreage needed to support one female BFF are 100-150 occupied acres (40-60 ha) based upon energetics of a captive female (Biggins et al. 1993, 2006*b*). The Recovery Plan tripled this number to one female per 375 acres for GPD as a conservative approach to address factors such as undercounting BFFs, climatic factors, poisoning, and disease. This adjustment is based upon lower estimates of BFF population density (one female per 216 acres) on BTPD colonies in Conata Basin, South Dakota (USFWS 2013). Tripling this number may be overly conservative. In addition, the Recovery Plan does not add a minimum occupied acreage for males as "they have overlapping ranges with female ferrets" (Biggins et al. 2006*b*, USFWS 2013).

Instead, for this plan, the Department has incorporated recent data on GPD densities in Arizona and BFF energetic requirements (Biggins et al. 1993) to calculate the number of GPD-occupied acres necessary to support BFF family groups.

The Department accepts a threshold of 10.1 active GPD burrows/acre to characterize "good BFF habitat" (Biggins et al. 1993). Seventy percent of all GPD-occupied habitat in the Aubrey Valley, Arizona, met or exceeded the threshold with an average of 26.8 active GPD burrows/acre. Based on the Biggins et al. (1993) formula to calculate animal densities from burrow densities, this

indicates an average in the Aubrey Valley of 3.93 GPD/acre (2007-2015, AGFD unpublished data) in "good BFF habitat." According to the energetics model in Biggins et al. (1993), the number of prairie dogs required to support one BFF family group is 763 per year.

Therefore:

GPD-occupied acres per BFF family group =

$$\frac{[GPD\ needed\ to\ sustain\ a\ BFF\ family\ group\ per\ year\ (i.e.\ 763\ per\ Biggins\ et\ al.\ 1993)] \times [GPD\ density\ in\ "good\ BFF\ habitat"]^{-1}}{percent\ of\ "good\ BFF\ habitat"\ in\ total\ GPD\ occupied\ acres}$$

Assuming Aubrey Valley, Arizona, averages are representative statewide, this formula equates to the following:

$$\frac{763 \times 3.93^{-1}}{0.70} \approx 277\ GPD - occupied\ acres\ per\ BFF\ family\ group$$

Therefore, the Department estimates that 277 GPD-occupied acres are needed to support one BFF family group (i.e. 1.5 breeding adults and 3.3 kits, Biggins et al. 1993), assuming a ratio of 2:1 females to males (Forrest et al. 1988, Livieri and Anderson 2012).

Therefore, to determine the GPD-occupied acres necessary to support target number of BFF family groups:

GPD-occupied acres to support target number of BFF family groups =

$$\frac{\text{GPD}-\text{occupied acres}}{\text{BFF family group}} \times Number\ of\ target\ BFF\ family\ groups$$

For 20 BFF family groups (i.e. 30 breeding adults and associated kits), a minimum of 5,540 GPD-occupied acres are required for a small population of BFFs. For Arizona to achieve 148 breeding adults (i.e. 99 family groups), with an assumed 2:1 ratio of 99 females and 49 males, the Department will maintain a minimum of 27,700 GPD-occupied acres, which is 81% of the 34,000 occupied acres identified in the Recovery Plan (USFWS 2013).

Using the above calculation, each of Arizona's BFF populations with a minimum of 30 breeding adults (composed of 20 females and 10 males) would require a minimum 5,540 GPD-occupied acres for a small population. As such, the Department will implement a standard for establishing a new BFF population in an area if it sustains, for three years, a minimum 5,540 GPD-occupied acres. After a BFF population is established, the Department will assess the GPD population periodically to determine an average GPD-occupied acreage for each location. Then, using the formula above the Department can determine an appropriate expected number of BFF family groups for each location.

As of 2015, only two known locations in Arizona meet the minimum GPD-occupied acre requirement for a small population (i.e. Aubrey Valley/Double O Ranch, and Espee Allotment).

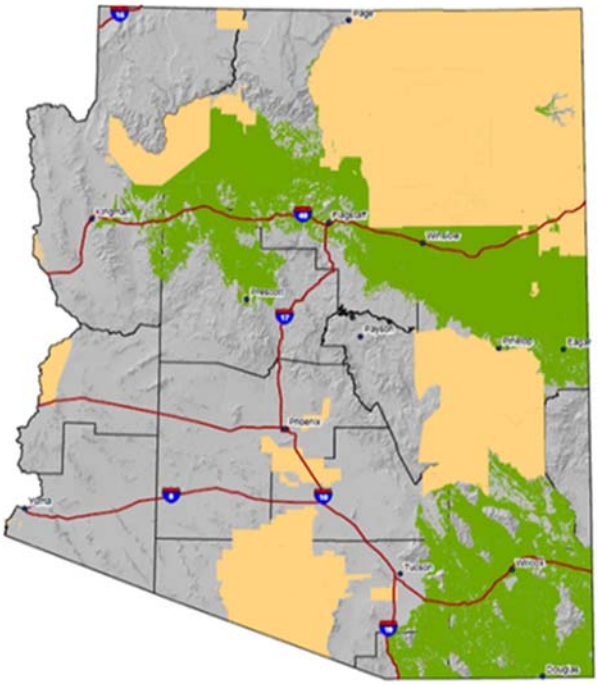

Figure 1. Grassland habitats (green) on public lands in Arizona with potential for BFF reintroduction.

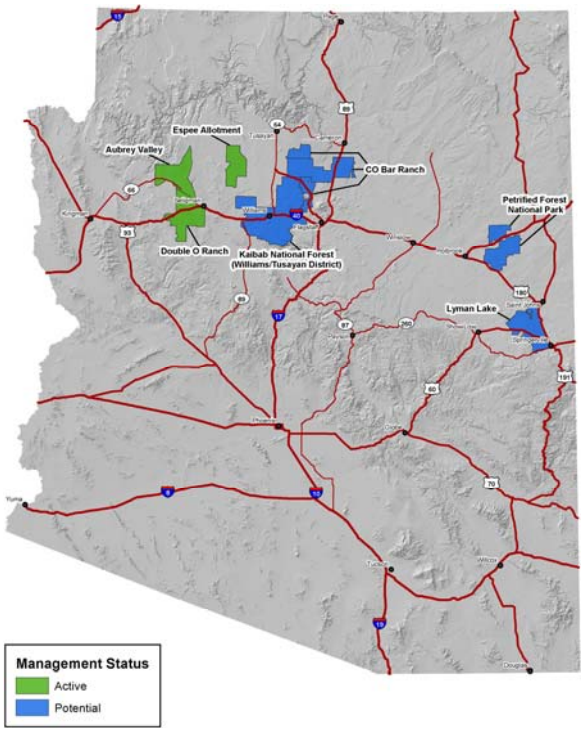

Figure 2. Potential areas for BFF populations in Arizona.

However, Arizona's public lands support a large amount of grassland habitat with varying sizes of dispersed GPD colonies (Figure 1). Alone, each of these discontinuous colonies may never reach the 5,540 GPD-occupied acres minimum requirement for a small population; together, however, these complexes may still support a BFF population if they are within the known dispersal distance of a BFF.

Forest et al. (1985) defines a prairie dog complex as a group of prairie dog colonies distributed so that individual BFF (and thus genetic material) can migrate among them commonly and frequently. Biggins et al. (1993) circumscribe prairie dog complexes with a distance based on the longest distance a BFF will travel in a single night. At Aubrey Valley, this distance is nine kilometers (AGFD unpublished data). Therefore, the Department will define colonies within nine kilometers, with no significant geographical or anthropogenic barriers, as part of the same complex.

Describing the locations for Arizona's proposed populations is subjective, but can be loosely based upon geographic or land ownership boundaries and organized by their potential to support a viable BFF population. Below we describe six potential areas for BFF populations based upon 2015 GPD population estimates. The Department will organize these areas into Active Management Areas (MA), Suitable MAs, and Potential MAs (Figure 2).

BLACK-FOOTED FERRET MANAGEMENT AREAS

Active Management Areas

Active MAs are areas where BFFs are released, managed, and monitored annually. When a BFF population exceeds the expected number of BFF family groups based upon the average GPD-

occupied acres using the formula above, and the population is stable or increasing for three or more years, the Department may translocate animals to other Active MAs or to Suitable MAs. When BFF populations are below the expected number of BFF family groups or the population is declining, the Department will evaluate translocations on a case-by-case basis.

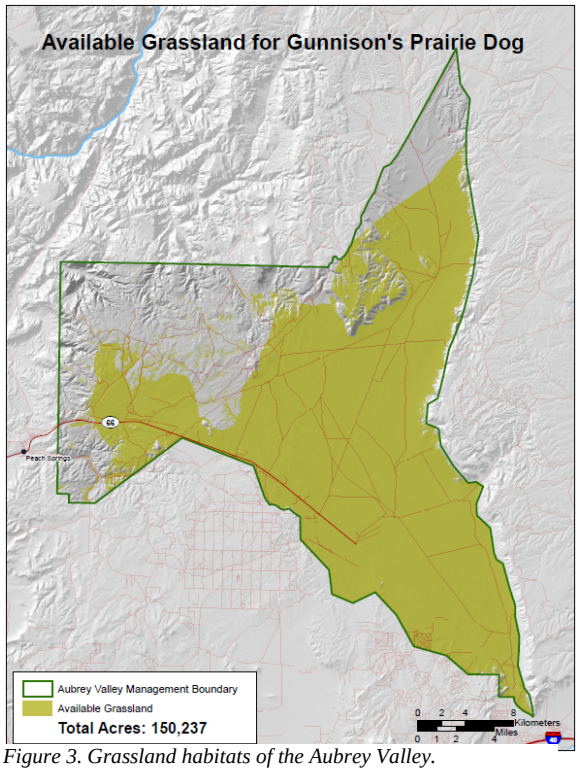

Figure 3. Grassland habitats of the Aubrey Valley.

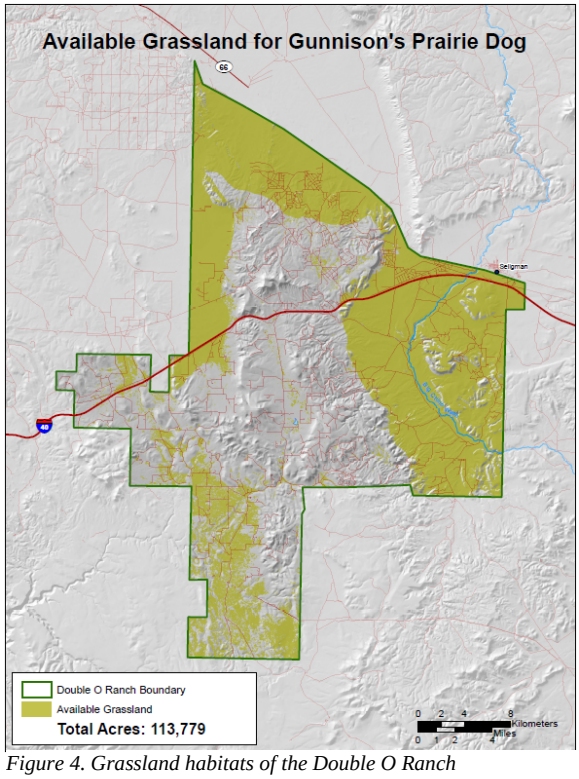

Figure 4. Grassland habitats of the Double O Ranch

Aubrey Valley/Double O Ranch – The Aubrey Valley encompasses 221,894 acres of private, tribal, state, and Bureau of Land Management (BLM) lands and is located approximately five miles west of Seligman in Coconino, Yavapai, and Mohave counties. The elevation ranges from 5,250-6,250 ft, and the annual precipitation is approximately 9.8-11.8 in. There are 150,237 acres of grassland habitat within the Plains and Great Basin Grassland and Great Basin Conifer Woodland biotic communities (Figure 3; Brown et al. 1979). The Double O Ranch encompasses 236,792 acres of private, state, and USFS lands south of the Aubrey Valley. The elevation ranges from 4,900-6,750 ft and the annual precipitation is approximately 9.8-11.8 in. There are 113,779 acres of grassland habitat within Plains and Great Basin Grassland and Great Basin Conifer Woodland biotic communities (Figure 4; Brown et al. 1979), of which there were 7,074 GPD-occupied acres known in 2014 (AGFD unpublished data).

Since 2005, GPD have occupied an average 48,542 acres (range 42,007 to 54,047) in the Aubrey Valley and have supported an average minimum of 67 BFFs. Based upon this average, and using the formula described above, the Aubrey Valley should support an average population of 175 BFF family groups (assuming 175 females and 87 males = 262 BFF breeding adults).

In 2015, GPDs occupy 45,905 acres of which 24,313 acres are in "good habitat." Since 2013, the number of GPD-occupied acres have been declining 8% annually, "good habitat" acres have been declining 22% annually, and the BFF minimum number alive has been declining 34% annually. The Department is unsure if this trend is due to emigration outside the Aubrey Valley, the

effects of drought, or some other unknown factor. Annual monitoring has not detected plague.

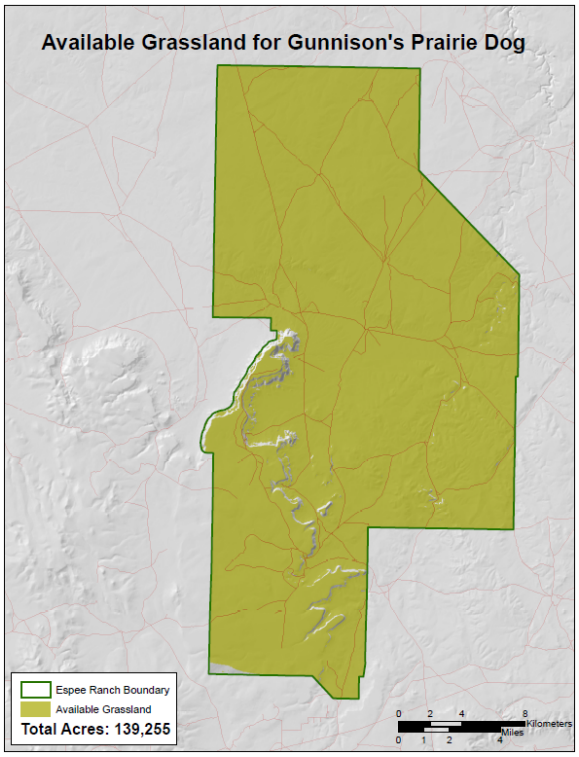

Figure 5. Grassland habitats in the Espee Allotment.

Espee Allotment – The Espee Allotment encompasses 145,644 acres of private and state lands approximately 50 miles northwest of Williams in Coconino County. The elevation ranges from 5,250-5,900 ft, and the annual precipitation is approximately 9.8-13.7 in. There are 139,255 acres of grassland habitat within the Plains and Great Basin Grassland biotic community (Figure 5; Brown et al. 1979), of which there were 3,228 GPD-occupied acres known in 2014 (AGFD unpublished data). Plague occurs on the Espee Allotment and is suspected for the lack of BFF observations despite multiple releases.

Suitable Management Areas

Suitable MAs meet, and have sustained for three years, the 5,540 minimum GPD-occupied acreage for a small population, and annual GPD monitoring occurs. As described above, Suitable MAs are ready to receive captive-raised and wild BFFs to establish new populations. Once in receipt of BFFs, a Suitable MA becomes an Active MA. Currently, there are no areas in Arizona that meet the minimum GPD-occupied acreage to be classified a Suitable MA.

Potential Management Areas

Potential MAs do not meet the minimum GPD-occupied acreage (i.e. 5,540 occupied acres) for a release, nor are they monitored annually. Management is necessary to improve GPD populations (i.e. translocations, dusting, or administration of the sylvatic plague vaccine), and annual monitoring of GPD populations must occur. Once a GPD population reaches and maintains the 5,540 minimum GPD-occupied acreage for a small population for three consecutive years, a Potential MA becomes a Suitable MA.

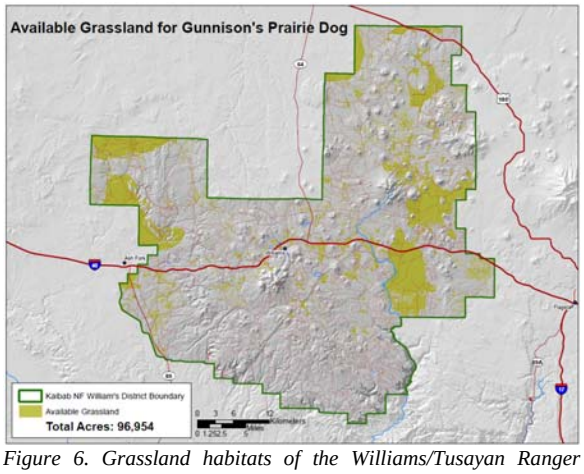

Figure 6. Grassland habitats of the Williams/Tusayan Ranger District, Kaibab National Forest.

Kaibab National Forest (Williams/Tusayan District) – The Kaibab National Forest, Williams/Tusayan District, encompasses over 613,000 acres of USFS, AGFD, Military, private, and state lands surrounding the city of Williams in Coconino and Yavapai counties. The elevation ranges from 4,600-10,200 ft, and the annual precipitation is approximately 8.6 in. There are 96,954 acres of grassland habitat within Plains and Great Basin Grassland, Great Basin Conifer Woodland, and Petrane Montane Conifer Forest

habitat biotic communities (Figure 6; Brown et al. 1979), of which there were 4,984 GPD-occupied acres known in 2015 (AGFD Unpublished Data).

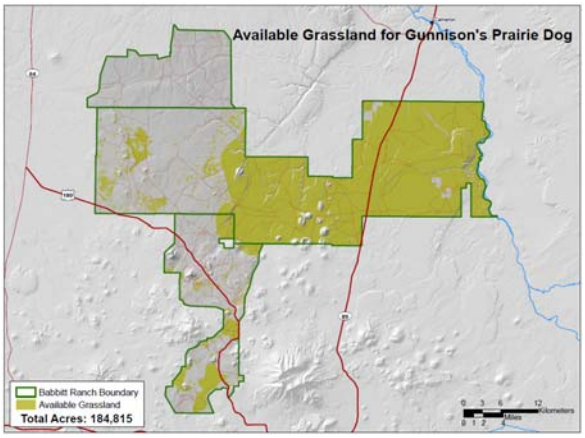

Figure 7. Grassland habitats of the CO Bar Ranch, Babbitt

CO Bar Ranch – The CO Bar Ranch encompasses 263,758 acres of BLM, state, private, and tribal lands and is approximately 24 miles north of Flagstaff in Coconino County. The elevation ranges from 4,200-7,400 ft, and the annual precipitation is approximately 9.8-18.8 in. There are 184,815 acres of grassland habitat within Plains and Great Basin Grassland, and Great Basin Conifer Woodland biotic communities (Figure 7; Brown et al. 1979), of which there were 870 GPD-occupied acres known in 2015 (AGFD unpublished data).

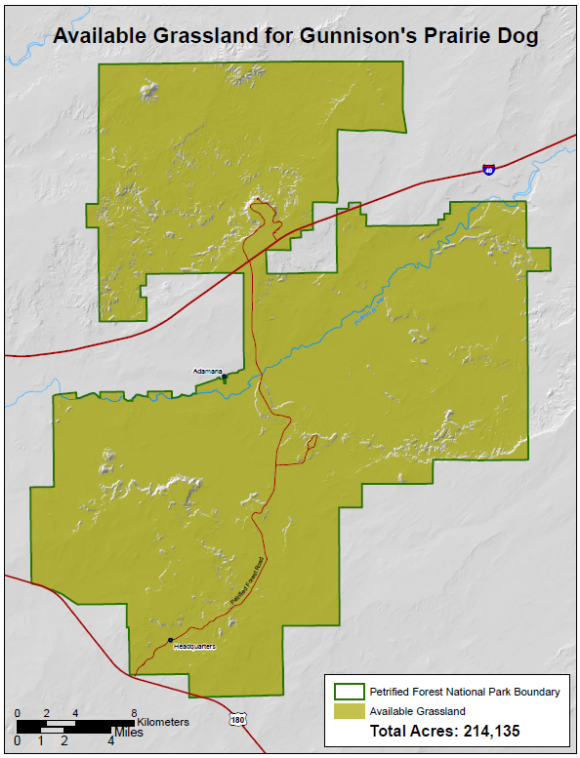

Figure 8. Grassland habitats of the Petrified Forest National Park.

Petrified Forest National Park – The Petrified Forest National Park encompasses 223,027 acres of NPS, BLM, state, private, and tribal lands east of Holbrook in Navajo and Apache counties. The elevation ranges from 5,250-6,250 ft, and the annual precipitation is approximately 10.4 in. There are 214,135 acres of grassland habitat within the Plains and Great Basin Grassland biotic community (Figure 8; Brown et al. 1979), of which there were 354 GPD-occupied acres known in 2015 (NPS unpublished data).

Lyman Lake – The Lyman Lake area encompasses 316,958 acres of private, state, AGFD, BLM, and USFS lands south of St Johns in Apache County. The elevation ranges from 5,800-9,600 ft, and the annual precipitation is approximately 9.8-11.8 in. There are 273,227 acres of grassland habitat within Plains and Great Basin Grassland, Great Basin Conifer Woodland, Great Basin Desertscrub, and Petrane Montane Conifer Forest biotic communities (Figure 9; Brown et al. 1979), of which there are 2,045 GPD-occupied acres known in 2015 (AGFD Unpublished Data).

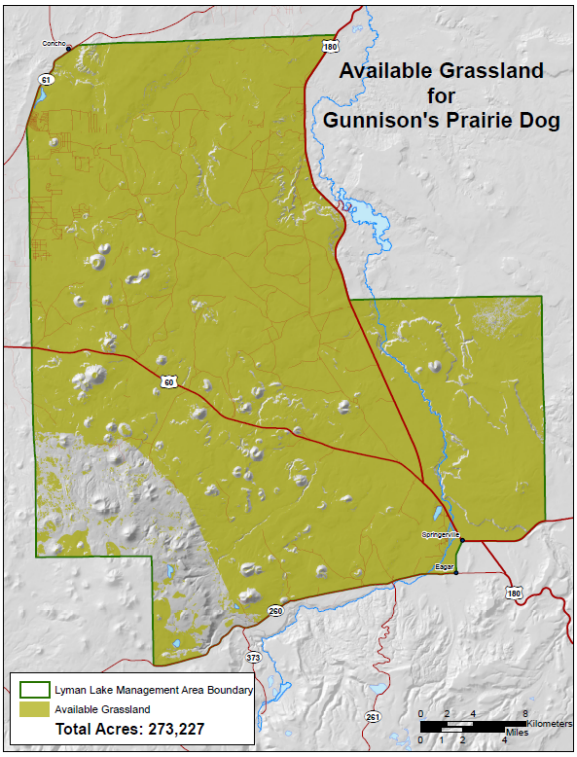

Figure 9. Grassland habitats of the Lyman Lake Management Area.

BLACK-FOOTED FERRET CONSERVATION AND MANAGEMENT STRATEGIES

Each location of a BFF population in Arizona will present unique challenges. Therefore, the Department may implement a variety of conservation and management strategies exclusively, or in combination, at each location while implementing this plan. These strategies will focus on both GPD populations to improve prey resources and availability for BFF, and on BFF populations to maximize potential growth and stability. Clearly, the viability and distribution of the Sylvatic Plague Vaccine may change how, and if, certain management strategies are implemented. For example, with an increase in GPD populations, GPD translocations may not be necessary, or GPD population monitoring could occur every three years instead of annually. However, until the USFWS' final approval of the Sylvatic Plague Vaccine, the Department will implement the following strategies to reach each area's expected number of BFF family groups. Each strategy described below may require the implementation of one or more protocols, which are below each strategy, and documented in detail in the Appendices.

GUNNISON'S PRAIRIE DOG MANAGEMENT STRATEGIES

Population Monitoring

Monitoring GPD populations allows the Department to detect changes in abundance, density, and distribution on local or statewide scales. The Department uses multiple methods to monitor GPD populations depending upon the need, scale, and intensity.

Perimeter mapping in small complexes, and correlated detection occupancy models for large complexes, allow the Department to determine the GPD-occupied acreage, to monitor the effects of drought, disease, and habitat changes, as well as the statewide stability of GPD populations. Once a GPD population or complex reaches the minimum GPD-occupied acreage for BFF reintroduction, a density mapping effort will estimate GPD abundance to inform reintroduction locations. Following a reintroduction, the Department will conduct density mapping annually for five years, and then reduce the effort to every third year. Unfortunately, density mapping is a labor-intensive process that does not provide immediate results. To assess the stability of a GPD population quickly (e.g. during a plague epizootic), the Department uses point count surveys. As needed, the Department will implement these surveys to monitor trends in the population between density mapping efforts.

Related Protocols:

- Gunnison's Prairie Dog Perimeter Mapping Protocol (Appendix A).
- Gunnison's Prairie Dog Correlated Detection Occupancy Models (Appendix B).
- Gunnison's Prairie Dog Density Mapping Protocol (Appendix C).
- Gunnison's Prairie Dog Point Count Survey Protocol (Appendix D).

Disease Monitoring

It is very difficult to detect epizootics of wildlife, especially those that are fossorial. None of the Department's disease monitoring techniques has ever detected an epizootic in sufficient time to prevent its spread or impact on GPD populations. To accomplish this, surveillance would need to occur daily on each population. This is logistically and financially unfeasible. Rather, the Department can monitor for the occurrence of disease, document new or re-emerging diseases, and develop management strategies to reduce their effects on the population through regular sampling of GPDs, small mammals, and predators.

Disease Surveillance – One mechanism to determine if disease is prevalent within or around any MAs is by collecting samples from carnivores, GPD, and other small mammals. While carnivores are resistant to plague and tularemia, serologic testing can identify recent exposure (Gage et al. 1994, Gese et al. 2004, Thrusfield 2007, Abbott and Rocke 2012). In addition, advanced tissue processing methods (i.e., polymerase chain reaction and immunohistochemistry) can detect organisms in the tissues of subclinically infected animals (Thrusfield 2007).

For GPDs and small mammals, the Department will sample twice annually (mid-spring and late summer) from the perimeter and opportunistically within two miles of an Active or Suitable MA. The number of trapping arrays for GPDs and small mammals will vary depending on the MA's size. The objective of the sampling strategy is to detect a plague outbreak before it enters a MA. Arrays will be randomly located around the perimeter of the MA and in habitat rated as good BFF habitat (i.e., 10.1 occupied burrows/acre). Trap arrays will consist of 100 live traps (75 Sherman for small rodents and 25 havahart for GPD) spaced 15 m apart on a 3 by 25 trap grid, except that havahart traps will be placed at active burrow entrances (Kraft and Stapp 2013). The number of arrays will be dependent upon the size of the MA with one array/2500 occupied acres and a minimum of one array/MA. This protocol may be modified to increase sample size and improve the probability of detecting plague at a 95% confidence level at prevalence of 10%. Blood (nobuto strips) and fleas will be collected from captured rodents and GPD for plague and additional disease testing. To avoid removing pregnant female GPD, lethal GPD sampling will not occur during the spring.

For carnivores (primarily coyotes but also foxes, and badgers), the Department will sample, or contract through USDA-APHIS Wildlife Services or an appropriate contractor, to conduct sampling twice annually (mid-spring and mid-fall) at opportunistic locations within four miles of an Active or Suitable MA (i.e., the home range radius for coyotes [Hibler 1977]). Timing is due to high antibody titers indicative of recent infection typically persist for four to eight months (Gage et al. 1994). The number of samples will vary depending on the size of MA. The objective will be to collect enough samples to detect a seroprevalence of 5% or greater with a 95% confidence based on the estimated population size of coyotes and other predators. During interepizootic periods in plague endemic areas seroprevalence is often 5% or less. A significant

increase above this baseline will be indicative of an impending or ongoing epizootic. Because predators, primarily coyotes, will not be present in large numbers compared to the prey animals, it may be difficult to obtain a significant sample size. For example, if the population of coyotes on a MA is 100 animals and 5% have been exposed to canine distemper or plague, then we would need to sample 45 animals to find a positive animal with a 95% confidence interval (Thrusfield 2007 pg. 239). The Department will also utilize hunter-harvested samples within six miles of the MA (average distance outside of home range for 70% of coyotes killed [Hibler 1977]). The Department may lethally remove and sample badgers (*Taxidea taxus*) within the MAs to increase disease detection.

Burrow Swabbing – The Department will conduct burrow swabbing to collect external parasites on Active MA twice annually and in response to a GPD population decline or suspected decline within an Active or Suitable MA. The Department will not conduct burrow swabbing at an abandoned GPD colony that has been vacant for longer than six months.

Related Protocols:

- Disease Surveillance Protocols for Sympatric Species (Appendix E).

Disease Management

Burrow dusting – Currently, the only mechanism to reduce the effects of plague on GPD populations is to dust burrows with a pyrethroid (e.g. deltametherin) to control fleas. However, dusting is logistically expensive, only locally effective, and current research shows fleas can develop resistance to pyrethroids with repeated use (Eads and Hoogland 2016). Therefore, the use of dusting as a management strategy should be limited to specific areas and for specific management purposes.

In a Potential MA, the Department may dust burrows to protect the GPD population from plague. In Active or Suitable MAs, the Department may dust burrows to prevent disease transfer between populations during translocation, and to ensure prey populations are viable to support BFFs.

To improve GPD population numbers in small or discontinuous colonies, the Department may dust twice annually (mid-spring and mid-summer) for a three to five year period. While there have been reports of flea populations developing resistance to pyrethroids, the factors which cause resistance are not completely understood and may relate to environmental parameters and not prior exposure (Boyer et al. 2014). To improve the success of GPD translocations and to prevent the spread of disease between areas, the Department's *Translocation Protocol for Gunnison's Prairie Dogs in Arizona* (Hicks et al. 2015) requires pre-translocation dusting at both the donating and receiving areas within two weeks of translocation. Similarly, to ensure a viable and healthy prey base for the initial reintroduction of BFF into a Suitable MA, the Department will dust burrows at a reintroduction area at least two weeks before the reintroduction of BFFs. Post-release, the Department will dust GPD burrows when a 10% decline in GPD numbers is detected in post-release monitoring.

Related Protocols:

- Burrow Dusting Protocol (Appendix F).

Translocations

GPD populations fluctuate in distribution and density due to a variety of factors. To release BFFs into a Suitable MA, the Department's minimum 5,540 GPD-occupied acres must be present for three consecutive years. In addition, areas may become unoccupied, creating discontinuous colonies that do not meet the nine-kilometer maximum distance requirement for a complex. To achieve or maintain these standards, GPD translocations may be necessary to repopulate abandoned areas or augment an existing population. In 2015, the Department produced a *Translocation Protocol for Gunnison's Prairie Dogs in Arizona* (Hicks et al. 2015). The Department will use this protocol to guide GPD translocations.

Seasonal Hunting Closures

When a GPD population declines, additive mortality from hunting pressure may lengthen the population's recovery time or may locally eliminate the population. Within Active MAs (either defined above or adaptively included), this may affect the GPD population's reproductive capacity and affect the Department's ability to reach or maintain the expected number of BFF family groups. Thus in Active MAs, the Commission may close GPD to hunting if monitoring (i.e., using one of the protocols identified above) shows a greater than 15% decline in GPD-occupied acreage over a three-year period. The Department will recommend eliminating closures when it is determined that the minimum level of GPD-occupied acres exceeds the level necessary to support existing BFF populations for three consecutive years based upon annual monitoring.

Supplemental Feeding

Supplemental feeding is a management strategy used to maximize reproductive potential and to reduce predation to GPD populations by providing necessary food and water at the burrows. Similar to dusting, this strategy is logistically expensive and only locally effective. Therefore the Department will only supplementally feed to improve GPD population size in small or discontinuous populations, after GPD translocations as described in *Translocation Protocol for Gunnison's Prairie Dogs in Arizona* (Hicks et al. 2015), and to ensure prey populations are viable to support reintroduced BFFs while they become established.

BLACK-FOOTED FERRET MANAGEMENT STRATEGIES

Population Monitoring

Mark and recapture via spotlighting is the primary method used to monitor BFF populations. This method allows for estimation of survival rates, reproduction, recruitment, and total population size. The Recovery Plan requires a high level of monitoring during establishment and for five years after the last BFF release (USFWS 2013). To accomplish this task, the Department will monitor BFF in Active MAs three times a year (mid-spring, mid-summer, mid-fall) in compliance with the Recovery Plan. Thereafter, reduced monitoring will ensure identification of trends in population demographics and the occurrence of surplus animals available for translocation into other MAs.

Each of the three monitoring periods assesses separate demographic events of the BFF population. Monitoring events in spring evaluate recruitment of the previous year's kits into the population and winter survival. Summer surveys assess reproduction as kits become active and

exit the natal den in mid-July. Fall surveys provide an opportunity to evaluate recruitment and estimate abundance.

Related Protocols:

- Black-footed Ferret Monitoring Protocol (Appendix G).
- Black-footed Ferret Capture and Handling Protocol (Appendix H).

Density Monitoring

As previously stated, estimates of the minimum GPD-occupied acreage to support one BFF family group in Aubrey Valley is 277 acres. Research has yet to identify if a smaller ratio is sustainable in GPD populations, or if there are density-dependent effects to the BFF or GPD population (i.e. increased predation, lower reproductive rates, etc.). However, using results of BFF and GPD population monitoring, the Department has the ability to evaluate if an Active MA is approaching or exceeding the Department's estimated requirement of one BFF family group per 277 GPD-occupied acres. If the estimated number of BFFs in an Active MA exceeds one BFF family group per 277 GPD-occupied acres, the Department will evaluate the opportunity to translocate animals to other MAs. Similarly, the Department will not supplement BFF populations in Active MAs with captive-bred BFFs or translocations if the density exceeds this rate.

Captive-bred Releases

All BFFs in North America are progeny derived from seven animals in a captive breeding program. Research on the survivorship of captive-bred BFFs has improved reintroduction protocols by developing captive environments so that BFFs learn to evade predators and hunt live prey (Miller 1988). Until populations recover to the expected number of BFF family groups where translocations are possible between MAs, the Department will rely on captive-bred animals to achieve statewide recovery.

As stated above, the Department will implement a standard for establishing a new BFF population if the MA sustains a required minimum 5,540 GPD-occupied acres for a small population for three years. The initial release will consist of 30 individuals at the 2:1 female to male ratio. To identify areas for releasing BFFs within a Suitable MA, the Department will use the GPD density mapping method and adhere to the USFWS Minimum Standards for BFF Releases (Black-Footed Ferret Recovery Implementation Team, in prep.). Within Active MAs, the Department will use the BFF and GPD population monitoring protocols included in this plan to determine the densities of both species. BFF releases will only occur if densities are below one BFF family group per 277 GPD-occupied acres, or where monitoring has shown a measureable gap in expected BFF occupancy.

Related Protocols:

- Black-footed Ferret Release Protocol (Appendix I).

Translocations

The translocation of wild born BFFs is the Department's preferred strategy to introduce or augment BFFs into Active or Suitable MAs. In Arizona, the use of wild born BFF in

reintroduction efforts has been more successful than naïve captive-bred BFF (AGFD unpublished data).

Prior to this plan, an area's expected number of BFF family groups was not established to quantify the availability of BFFs for translocation efforts, nor were minimum release numbers, sex ratios, or a minimum required prey base. The Department will consider translocation if: 1) the source Active MA exceeds the expected number of BFF family groups for the area or densities exceed one BFF family group per 277 GPD-occupied acres, 2) the population is stable or increasing, and 3) the number removed will not cause the population to fall below the expected number of BFF family groups for the area. Unfortunately, the likelihood of a single Active MA having a surplus of 30 BFF at the appropriate sex ratio to reintroduce into a Suitable MA is unlikely. However, it is more likely the Department will remove a smaller number of surplus animals from multiple Active MAs in order to reach the minimum 30 animals (at the appropriate sex ratio). More frequently, the Department will use translocation as a strategy to augment populations and to fill occupancy gaps within Active MAs.

As a standard, the Department will translocate animals from the highest densities within the source population to avoid creating a local sink or occupancy gap in the population. In addition, the Department may capture BFFs in the fall, and place them in pre-conditioning pens in order to facilitate the release of pregnant females in the spring.

Captive Breeding

Nationally, the status of the BFF population has been trending downward; however, participating States continue to establish new reintroduction areas, which increases the demand on existing breeding facilities to supply the States' with releasable animals. An alternative to possible delays resulting from low BFF availability from existing national facilities is to develop a captive breeding program and/or pre-conditioning pens in Arizona. Biggins et al. (2006) suggested that post-release survival of adult BFF might be increased if animals were given earlier and longer exposure to the quasi-natural environments of pre-conditioning pens. Thus, providing animals the opportunity to adjust to local environmental conditions in pre-conditioning pens prior to release may enhance survival and success of captive-born BFF.

In 1997, Arizona's Aubrey Valley was the first BFF release area to begin an on-site breeding program in outdoor pre-conditioning pens. From 1997-2000, the Department successfully released 26, 63, and 29 kits respectively (Winstead et al. 1999, 2000, 2002a). In 2001, the Department modified the strategy to release mid-gestation BFF females to give birth in the wild. In 2001 and 2003 to 2006, the Department released 4, 6, 11, 9, and 6 females respectively. Through this effort, the Department documented that the release of gestating female BFFs in the spring increased kit survival rates. In spring, GPD pups are an abundant food resource that allows for reduced predation risk and greater energy conservation to pregnant BFFs. In addition, BFF kits can practice their hunting skills on the young and inexperienced GPD pups.

Establishing and maintaining a captive breeding program is logistically expensive, and the Department discontinued its program in 2007. Before investing in a renewed captive breeding program, the Department will perform a cost-benefit analysis. Maintaining a breeding program requires daily maintenance of fences and burrows, pen cleaning, and obtaining prey to feed

captive BFFs. An operational BFF captive breeding facility will require dedicated on-site personnel, the resources to support the effort, and a new centralized location on a GPD colony to minimize travel distance to Active MAs. However, the benefits of having a local supply of acclimated BFFs for release should shorten the time to reach the expected number of BFF family groups and state specific targets in each MA. In addition, the Department can research the survivability of BFFs bred on site as compared to those that derive from another location.

Related Protocols:

- Black-footed Ferret Breeding Protocol (Appendix J).

Health Monitoring

Detection of health issues affecting species can be very difficult, especially in nocturnal species and those that spend the majority of their lives underground such as BFF. Assessment of health status is an adaptive process and can only be successful with regular collection of data. One approach to health assessment and monitoring is to gather data on members of the population whenever possible. BFF monitoring (via mark and recapture) provides an opportunity to collect samples and assess baseline values of physiologic parameters, and exposure to disease. In addition, active health monitoring may allow for detection of disease events in advance of widespread mortality. The Department will collect the samples listed in Table 1 for whenever BFF are captured during population monitoring events. We will evaluate the BFF population(s) for the development of genetic bottleneck, heritable diseases (e.g. renal amyloidosis), and exposure to toxicants (lead and rodenticides e.g.) and infectious diseases.

Table 1. Black-footed ferret samples collected for health monitoring.

Sample	Handling	Testing
Hair	Paper envelope	Genetic
Feces	Whirl pack or plastic vial, refrigerate	Parasitology (float, direct); virology, bacteriology
Blood	Blood tubes (EDTA, Tiger top, capillary tubes), process immediately or refrigerate	Hematology and clinical chemistry (on site ISTAT, or diagnostic lab), DNA, serology.
Fleas, ticks, lice	Place in alcohol or saline	Screen for bacteria, viruses.

Predator Management

The primary predators of BFFs are badgers, bobcats (*Lynx rufus*), coyotes (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), kit fox (*Vulpes macrotis*), and raptors. To characterize the relative densities of predator populations occurring within Active and Suitable MAs, the Department will conduct predator index surveys during BFF population monitoring and mark-recapture events in spring and fall and one month prior to any translocation or release. These surveys will enable the Department to document trends in predator densities and inform decisions regarding whether predator management is appropriate to support and promote a viable BFF population.

Prolonged predator control is biologically ineffective and logistically prohibitive (Breck et al. 2006). However, if BFF population estimates decrease, the Department may investigate options to manage predation and promote BFF population growth. Once a MA achieves the expected number of BFF family groups, the Department will reduce efforts to manage predation, and monitor the BFF population to ensure sustainability.

The Department will conduct predator management as necessary. Predator management prior to releasing naïve BFFs may allow time for dispersal and the establishment of home ranges. Increased predator management during the summer and fall may also allow BFF kits safer opportunities to learn to hunt and time to disperse. Until a MA achieves the expected number of BFF family groups, the Department may opportunistically remove badgers, coyotes, and foxes within the MA as these species can prey on GPD and BFF.

Related Protocols:

- Predator Population Trend Protocol (Appendix K).

ADAPTIVE MANAGEMENT

As with any wildlife management plan, the Department's ability to manage adaptively is critical to meet the objectives. Environmental variation, local habitat conditions, and threats to sustainable wildlife populations are constantly changing. While this management plan identifies mechanisms and strategies to achieve statewide recovery, future research will improve biological knowledge, and develop new tools (i.e., Sylvatic Plague Vaccine) to aid in conservation that may date the information contained herein. Similarly, the MAs proposed are not exhaustive. The Department will prioritize new areas and willing landowners to hasten population growth. The Department will use monitoring, research, and partnerships to ensure the use of the best knowledge, strategies, and MAs to reach statewide recovery efficiently and effectively.

REVISION OF THE NONESSENTIAL EXPERIMENTAL POPULATION DESIGNATION

PURPOSE

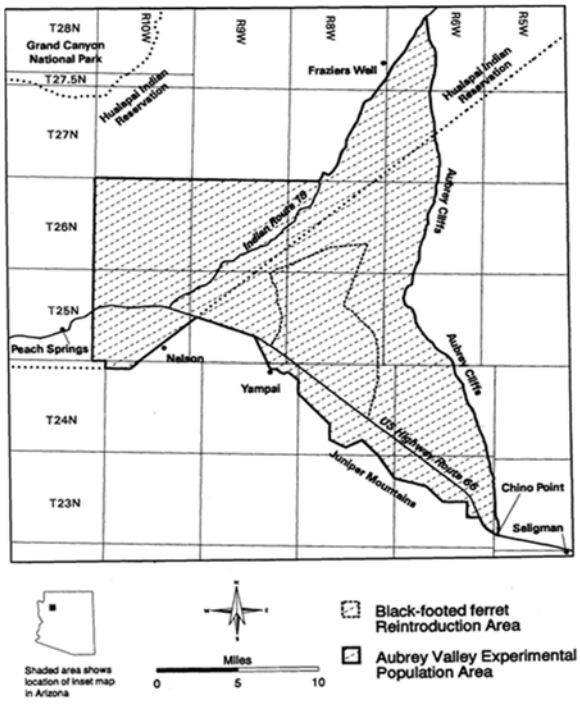

Figure 10. Nonessential Experimental Population designated area in Aubrey Valley, Arizona.

As stated in the Introduction, the only mechanism to establish an Arizona BFF reintroduction program with broad public support in the 1990s was through the establishment of Nonessential Experimental Population Designation as prescribed in Section 10(j) of the ESA (Figure 10). This designation has allowed the Department to research and manage the reintroduced population as a threatened species as allowed under Section 6 of the ESA (50 CFR § 17.21 and 17.31), and has allowed landowners the ability to continue land use practices without BFF having the full legal protection of the ESA.

Through this plan, we proposed to manage multiple BFF populations statewide on lands owned, managed, or leased by federal, state, local agencies, tribal, and private individuals. To receive the same public support to implement this

statewide plan, the Department will seek a revision to the Nonessential Experimental Population Designation across all appropriate biotic communities (grassland habitats) within Arizona (Figure 11).

RECOMMENDED REVISED RULE

The current rule contains the following information. The proposed Department changes are italicized.

§ 17.11 Endangered and threatened wildlife.
(h)

Species		Historic range	Vertebrate population where endangered or threatened	Status	When listed	Critical habitat	Special rules
Common name	Scientific name						
MAMMALS							
Ferret, black-footed.	Mustela nigripes.	Western U.S.A., Western Canada, Mexico.	Entire, except where listed as an experimental population.	E	1, 3, 433, 545, 546, 582, 646, 703, 737, 860	NA	NA
Ferret, black-footed.	Mustela nigripes.	Western U.S.A., Western Canada, Mexico.	U.S.A. (WY and specified portions of AZ, CO, MT, SD, and UT, see 17.84(g)(9)).	XN	433, 545, 546, 582, 646, 703, 737, 860	NA	17.84(g)

■ 3. Amend § 17.84 by:
■ a. Revising paragraphs *(g)(6)(iv), and (g)(9)(iv*);
■ c. By replacing the map entitled "Aubrey Valley Experimental Population Area" with a map entitled "Arizona Black-footed Ferret Nonessential Experimental Population Area."

The revisions and additions read as follows:

§ 17.84 Special rules—vertebrates.

(g)(6)(iv) Report such taking in the *Arizona experimental population area* must be reported to the Field Supervisor, Ecological Services, Fish and Wildlife Service, Phoenix, Arizona, telephone (602) 640–2720.

(9)(iv) The *Arizona Experimental Population Area* is shown on the attached map for Arizona and will be considered the core recovery areas for this species *in Arizona*. The boundary of the *northern* nonessential experimental population area will be those parts of *Apache, Coconino, Gila, Mohave, Navajo, and Yavapai counties that include the areas east of Arizona State Highway 66 between Interstate 40 and the Hualapai Indian Reservation; south and east of the Hualapai Indian Reservation to the Colorado River; south of the Colorado River excluding the Havasupai Indian Reservation to the Navajo Indian Reservation; west and south of the Navajo Indian Reservation to the Arizona State border with New Mexico; west of the Arizona State border with New Mexico to the southern boundary of Apache County; north of the southern boundary of Apache County to the White Mountain Indian Reservation; east and north of the White Mountain Indian Reservation to Arizona State Highway 260 near Pinetop, Arizona; north of Arizona State Highway 260 between Pinetop, Arizona and Interstate 17 at Camp Verde,*

Arizona; north of Interstate 17 between Camp Verde, Arizona and Arizona State Highway 69; north of Arizona State Highway 69 between Interstate 17 and Arizona State Highway 89; north of Arizona State Highway 89 to Arizona State Highway 93; and north of Arizona State Highway 93 to Interstate 40. The boundary of the southern nonessential experimental population area will be those parts of Cochise, Pima, Pinal, Graham, and Santa Cruz counties that include the areas east of Interstate 19, Interstate 10 and Arizona State Highway 77 from Nogales, Arizona to the southern boundary of the San Carlos Apache Tribal Lands; south of the southern boundary of the San Carlos Apache Tribal Lands to the southern boundary Greenlee County; south of the southern boundary of Greenlee County to the Arizona and New Mexico border; west of the Arizona and New Mexico border to the Arizona and Mexico International border; north of the Arizona and Mexico International border to Nogales, Arizona. Any black-footed ferrets found in the wild within these boundaries will be considered part of the nonessential experimental population after the first breeding season following the first year of releases of ferrets into the reintroduction area. A black-footed ferret occurring outside the experimental area in Arizona would be considered as endangered but may be captured for genetic testing. Disposition of the captured animal may take the following action if necessary:

(A) If an animal is determined to have originated from the experimental population, either genetically or through tagging devices, it may be returned to the reintroduction area or to a captive facility. If a landowner outside the experimental population area wishes to retain black-footed ferrets on his property, a conservation agreement or easement may be arranged with the landowner.

(B) If an animal is determined to be genetically unrelated to the experimental population, then under an existing contingency plan, up to 1% of the ferrets may be taken for use in the captive-breeding program. If a landowner outside the experimental population area wishes to retain black-footed ferrets on his property, a conservation agreement or easement may be arranged with the landowner.

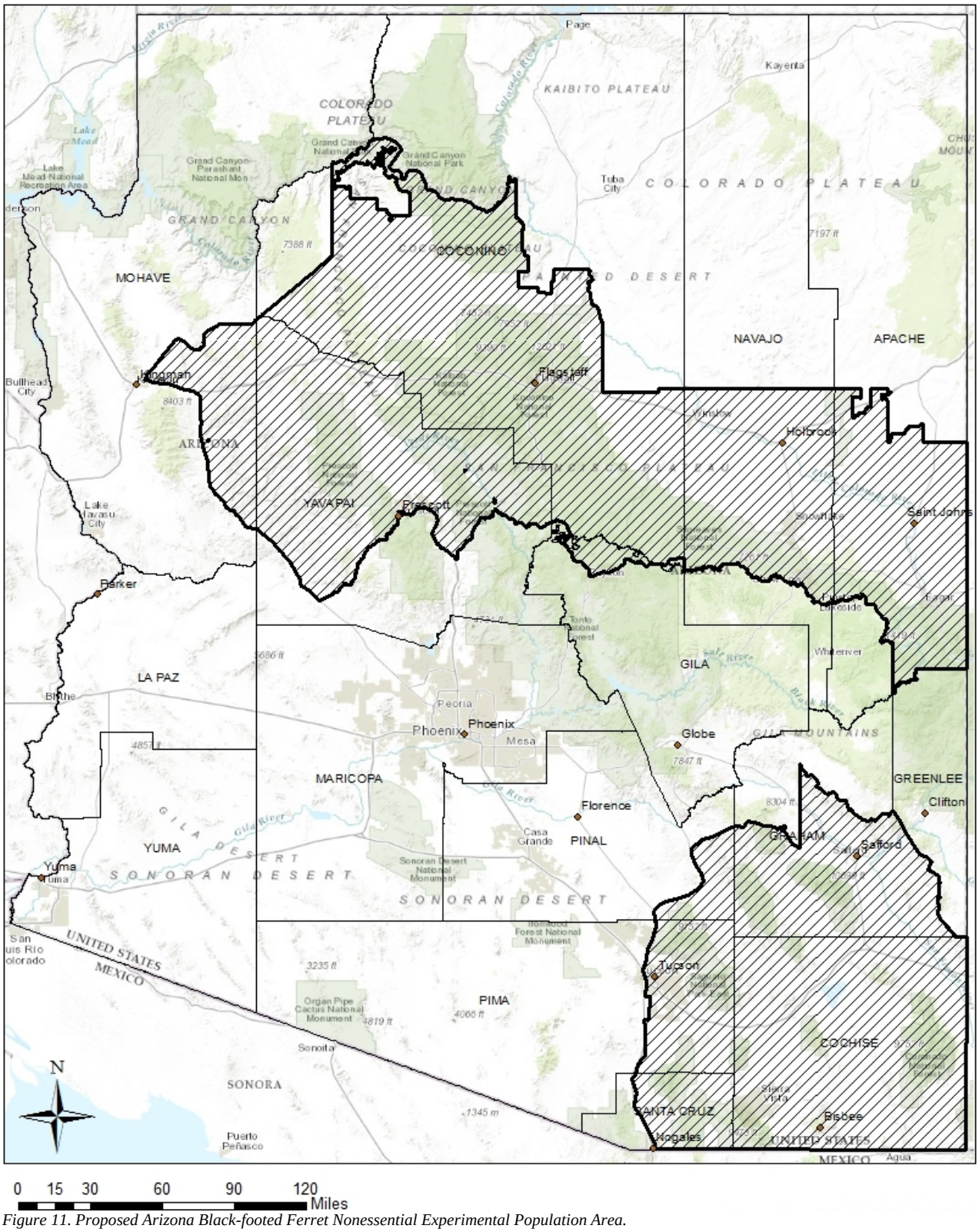

Figure 11. Proposed Arizona Black-footed Ferret Nonessential Experimental Population Area.

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APPENDIX A: GUNNISON'S PRAIRIE DOG PERIMETER MAPPING PROTOCOL

PURPOSE

This protocol provides guidance to determine occupied acreage of prairie dog colonies. Gunnison's prairie dog (*Cynomys gunnisoni*) population estimates are difficult to obtain; therefore, status of the prairie dog population is evaluated by the acreage they occupy.

BACKGROUND

An occupied colony of prairie dogs has large mounds and indications of activity (e.g. fresh scat and digging). To determine the amount of acreage the prairie dogs occupy, outlining the perimeter of the colony delineates the occupied acreage of the colony. To determine density and an estimate of the population size, this method is used in conjunction with visual counts or density mapping.

PROTOCOL

EQUIPMENT

1. GPS
2. Binoculars.

FIELD PROTOCOL

1. Locate the outer edge of the colony, and turn on the tracks function on the GPS.
2. Walk around the outer edge of the colony using binoculars to scan ahead and around to ensure all active burrows are within the perimeter.
    a. To determine whether a burrow is active, turn off the tracks function and mark a waypoint. Walk to the burrows in question and determine if it is active. Once it has been determined whether those burrows should be included, walk back to the waypoint, turn tracks back on, and resume walking the perimeter.
        i. An alternative would be to conduct a preliminary survey of the site to determine active burrows prior to beginning the track.
3. Turn off the tracks function and save the track.
4. The saved track should display the acreage within the perimeter.
    a. An alternative is to upload the perimeter onto a computer and determine acreage using computer software (e.g. GPSExpert or ArcGIS).

APPENDIX B: GUNNISON'S PRAIRIE DOG CORRELATED DETECTION OCCUPANCY MODELS

PURPOSE

This protocol reviews methods for surveying prairie dog habitat and recommends using correlated detection occupancy models to estimate the number of occupied acres. This approach requires surveying rectangular plots composed of 1-acre subplots for visual observations of prairie dogs. Data analysis will account for imperfect detection and estimate occupied acres.

BACKGROUND

Methods to estimate occupied acres of prairie dog habitat are needed as this metric is a key recovery criterion for the endangered black-footed ferret (*Mustela nigripes*; hereafter BFF) (USFWS 2013). However, defining and measuring occupied acres have both proven to be difficult tasks (McDonald et al. 2011). When defining occupied acres, there is uncertainty about how to treat foraging habitat, how to count areas of low prairie dog density, and the appropriate scale of measurement. For example, it is not clear from the Recovery Plan if occupied acres are limited to acres of active burrowing habitat, or include foraging habitat, which may extend beyond burrows. Black-tailed prairie dog (*Cynomys ludovicianus*; hereafter BTPD) colonies are frequently delineated by a foraging "clip" line that is relatively visible (Odell et al. 2008), but white-tailed (*Cynomys leucurus*) or Gunnison's prairie dog (*Cynomys gunnisoni*; hereafter GPD) colonies are more typically delineated by the limits of burrows due to a lack of a clear clip line (Biggins et al. 1993). Furthermore, occupancy has been variously defined to exist at minimum densities of 10 burrows/ha (Forrest et al. 1985), 20 burrows/ha (Biggins et al. 1993), and one prairie dog per acre (Peek et al. 2014). The scale of measurement can significantly affect estimates of occupied acres, but established standards are lacking (McDonald et al. 2011). At one extreme, an instantaneous and error-free survey of square-foot survey units would find that very few acres are occupied. In this case, a map of occupied space might look like stars in the sky, with tiny occupied spaces scattered in a large unoccupied matrix. If the scale of measurement is increased to one square mile, then a single prairie dog could be recorded as occupying 640 acres, even though it may never use 639 of those acres. Various standards for the measurement scale have been proposed, including one acre (Peek et al. 2014) and different measures based on home range sizes (McDonald et al. 2011). A common alternative is to create minimum convex polygons circumscribing colonies (Biggins et al. 1993). With polygons, the measurement scale is imposed by the survey protocol but not explicitly defined. For example, documented or undocumented decisions about acceptable distances between burrows affect the border of polygons and the delineation of interior holes within polygons.

Even if a definition of occupied acres is accepted, determining the presence/absence of prairie dogs can be difficult. As fossorial animals, prairie dogs are often below ground and may be difficult to observe. Burrows are typically much easier to observe, but burrows may be unoccupied for years, so acres with burrows may overestimate acres occupied by prairie dogs (McDonald et al. 2011). A common approach to identifying occupied burrows is to search for fresh scat (Biggins et al. 1993). However, the reliability of scat as an indicator has not been

rigorously investigated, and plague outbreaks may result in areas with fresh scat and no prairie dogs (McDonald et al. 2011).

ALTERNATIVE SURVEY METHODS

One common approach to estimating occupied acres of prairie dog habitat is to map colonies (Biggins et al. 1993). In mapping, there is an effort to delineate colony boundaries with a polygon, and then calculate the area encompassed by the polygon. Mapping may be done from plane surveys or satellite imagery, or from ground surveys. Although boundary mapping is commonly used for BTPDs, mapping GPD colonies is more difficult due to indefinite colony boundaries and more visually complex habitat types (Andelt et al. 2009, McDonald et al. 2011). Furthermore, ground surveys are labor intensive on large colonies and have the potential for errors. For example, a surveyor may not detect a large unoccupied area (or "doughnut hole") in the middle of a colony while walking its perimeter. Aerial surveys may reduce labor costs on large colonies, but they have a reduced ability to distinguish active and inactive burrows (McDonald et al. 2011). Accordingly, this approach has been described as inadequate for GPD (Andelt et al. 2009, McDonald et al. 2011).

An alternative approach that has been used for GPD is occupancy estimation (Andelt et al. 2009, McDonald et al. 2011, Seglund 2012). In this approach, plots of land are surveyed on multiple occasions for evidence of prairie dogs. In recent applications, plots have measured 500 m × 500 m (62 acres), and evidence of occupancy has been obtained by visual observation of prairie dogs (Andelt et al. 2009). Occupancy surveys are appealing because there is a well-developed statistical theory that supports estimates of uncertainty (MacKenzie et al. 2006). For large study areas, valid estimates of the proportion of plots that are occupied can be obtained by surveying a representative portion of the study area, rather than attempting to map the entire area. Furthermore, occupancy surveys use methods to account for imperfect detection of animals during surveys, an important consideration for fossorial animals (MacKenzie et al. 2006). However, plots of 62 acres may not be an appropriate scale for estimating occupied acres because prairie dogs may only use a fraction of the plot, generating "rounding up" errors (McDonald et al. 2011). It is theoretically possible to reduce rounding up errors by surveying a larger number of smaller plots. However, this would increase labor costs, especially when each plot must be visited twice.

Here, we propose adopting an alternative method. Newly developed correlated-detection occupancy models (Hines et al. 2010, 2014) are designed to estimate occupancy from adjacent subplots. Surveying several adjacent subplots reduces travel costs associated with each plot. Under traditional occupancy modeling, spatial correlation between adjacent subplots would violate model assumptions (MacKenzie et al. 2006). However, correlated detection occupancy models can account for this correlation (Hines et al. 2010). Furthermore, by using spatial replication to inform detection probabilities, it is only necessary to visit each plot once. Traditional occupancy modeling estimates the proportion of large plots that are occupied, but in correlated-detection models, we can derive an estimate of the proportion of subplots that are occupied. If the selected plots are representative of the study site, then the estimate of occupied acres can be extrapolated to the entire study area. Therefore, surveying adjacent 1-acre subplots for visual encounters of prairie dogs and analyzing the data with correlated-detection occupancy

models could reduce rounding up errors and reduce labor costs while generating a defensible estimate of occupied acres.

A correlated detection occupancy model requires dividing a study area into rectangular plots composed of adjacent 1-acre subplots, randomly selecting a sample for surveys, surveying each subplot for presence/absence of prairie dogs (via visual observation), and analyzing survey data to estimate occupied acres. This process is detailed below.

Selection of Areas to Survey

Selecting areas to survey requires the following steps:

1. Define the population of interest.
2. Define a feasible sampling frame.
3. Delineate sample units.
4. Select sample units.

Population of Interest

Prior to designing a survey, it is necessary to define the population of interest. This is the collection of individuals about which information is desired. This might be all acres of potential prairie dog habitat within an isolated grassland or within an arbitrary boundary. Once the population of interest is identified, surveys should be designed to gain information about this population (see Box 1).

Sampling Frame

A sampling frame divides the population of interest into a comprehensive list of identifiable and observable elements. In the case of prairie dog habitat, the sampling frame could consist of a comprehensive list of acre plots covering the population of interest. However, if the population of interest is partially on inaccessible land, the sampling frame may cover only a portion of the population. In this case, the strongest inference will be limited to the sampling frame, not the entire population. If the population has the potential to move during the course of a planned monitoring program, it is advisable to expand the sampling frame to cover potential habitat from the beginning of the program.

Delineate Sample Units

At large study sites, it is not possible to survey the entire sampling frame. In this case, the sampling frame should be divided into smaller sample units, some of which will be selected for surveys. A 64 m × 64 m plot is approximately an acre, so that a plot of 64 m × 640 m would contain 10 1-acre subplots, while a 64 m × 1280 m plot would contain 20 1-acre subplots. Plots of this size could be organized into a regular grid covering the population of interest. Plots truncated by the edge of the study area will be smaller. To avoid surveying small plots, plots smaller than half the intended size could be eliminated from subsequent selection.

Select Sample Units

To draw conclusions about the population of interest, it is necessary that all sample units within the sampling frame have a known and non-zero probability of being selected for sampling. In

contrast, in convenience sampling (such as when sampling only along roads), some sampling units (such as those away from roads) will have zero probability of being selected. While convenience sampling can be attractive because it can reduce costs and increase sample sizes, it is vulnerable to bias, samples cannot be generalized to the population of interest, and it is not possible to estimate variances. For these reasons, probability-based sampling is preferred.

Box 1: Sampling Concepts

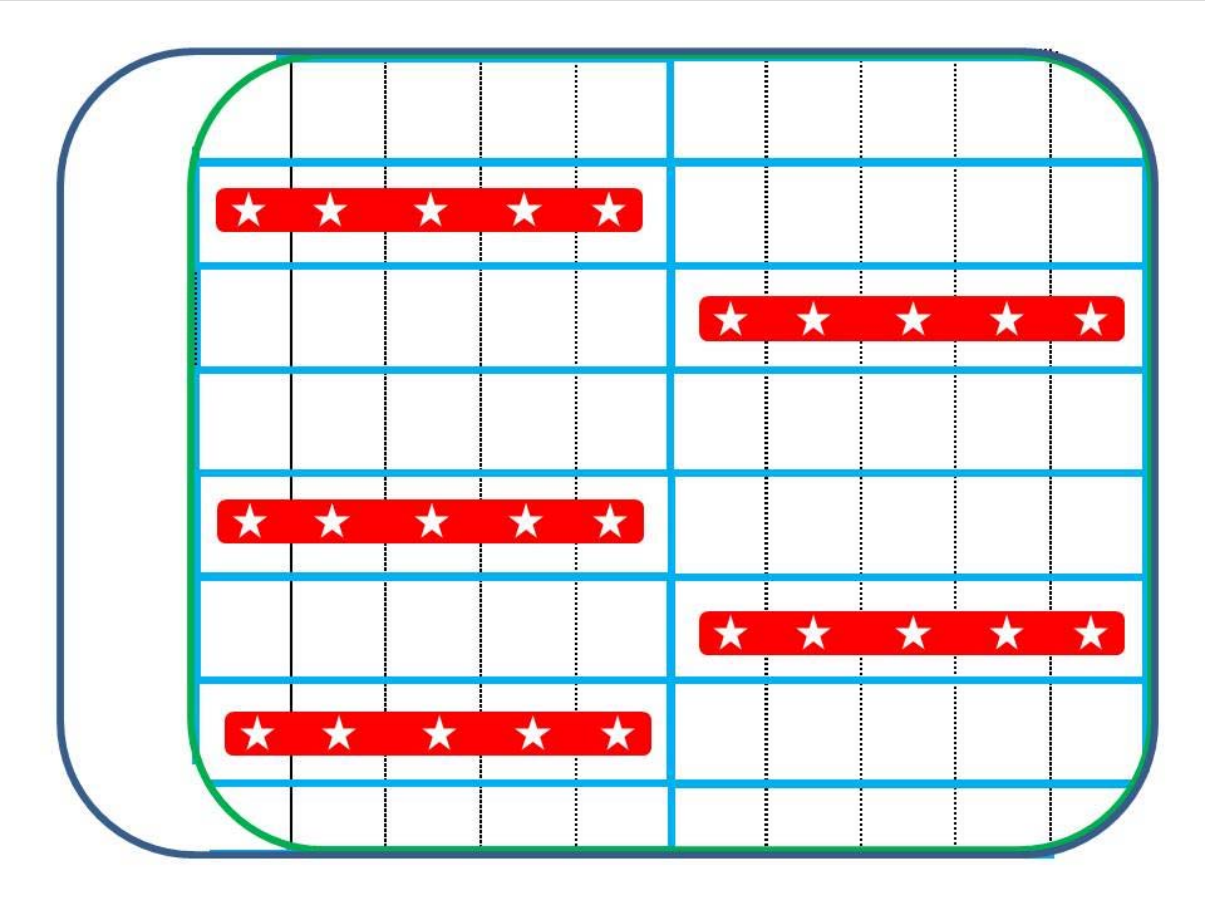
Population: All prairie dog habitat of research interest. Contained in dark blue shape.

Sampling Frame: Population organized into a comprehensive list. Contained in green shape. A portion of the population was excluded from the sampling frame.

Sample Units (Plots): Individual plots within the sampling frame that could be selected for surveys. Each light blue rectangle is a sample unit. Sum of all sample units equals the sampling frame.

Subplots: Each plot is divided into subplots, denoted by dotted black lines. If a plot is selected for surveys, each subplot will be surveyed for prairie dogs.

Sample: The sample units randomly selected for surveys. The red bar indicates a plot was selected for survey, while the stars indicate that each subplot is surveyed for prairie dogs.



Generalized random tessellation sampling (GRTS) is an effective tool for selecting a probabilistic sample from a sampling frame (Stevens and Olsen 2004). GRTS selects sample units by applying systematic random sampling to a hierarchical spatial tessellation of sample units. This approach generates better spatial balance than simple random samples, which reduces sampling error and improves model-based inferences. GRTS also provides more accurate variance estimates than systematic sampling. Another advantage of GRTS sampling is that it is scalable, so that if survey effort increases or decreases through time, the sample size can be adjusted without losing spatial balance.

Using GRTS in Program R requires a shapefile that describes the sample units (including location, strata, and weighting), and familiarity with R package 'spsurvey'. Properly specified, the 'grts' function will return a list of sample units to be surveyed. To make the sample scalable, R can be instructed to generate an ordered list of all sample units. In this case, if survey resources increase or decrease, the first *n* sample units can be selected, and the sample will remain random and spatially balanced.

Existing Data and Simulations Used to Inform Development of the Protocol

The optimal survey design depends on the characteristics of the population under study. For example, different protocols will be optimal when detection probabilities are high or low. Therefore, information about the population under study can help improve survey design. For correlated-detection occupancy models, the most important parameters are occupancy, detection probability, spatial correlation, and survey effort. Therefore, we obtained information on these parameters relevant to prairie dog surveys from the scientific literature and from previous prairie dog surveys conducted by the Arizona Game and Fish Department (Department). We then used these parameters in a simulation study to inform survey design. Our simulations allowed us to examine resulting accuracy, bias, and precision of various sampling designs.

Occupancy

Occupancy is the portion of plots (not subplots) that are occupied by at least one prairie dog. A brief review of previous prairie dog mapping efforts by the Department indicated that in areas with BFFs, prairie dogs are widely present. Therefore, we used occupancy rates of 0.75 and 0.90 in the simulations.

Detection

Detection is the probability of detecting a prairie dog, given that it is present. Detection for GPD in Colorado was estimated to be 0.79 for 500 m × 500 m plots with four 5-minute point counts (Andelt et al. 2009). Given the different survey methods proposed here, detection rates could vary. The small subplots are easier to search, which may increase detection. However, a small subplot may be more susceptible to disturbance, driving prairie dogs underground, and decreasing detection. Furthermore, we propose surveying each subplot for less than five minutes, which may lower detection. Considering these factors, we used detection rates of 0.4 and 0.6 in simulations, although there is considerable uncertainty about the true detection rate. Given the uncertainty, it is appropriate to assume relatively low detection probabilities when designing surveys (Clement, in press).

Spatial Correlation

Correlated-detection occupancy models include two correlation parameters, which indicate the probability that a subplot is occupied given the previous subplot is *unoccupied* and the probability that a subplot is occupied given the previous subplot is *occupied*. A review of density maps created by the Department indicated that areas occupied by prairie dogs were adjacent to other areas occupied by prairie dogs, suggesting that spatial correlation was high. Therefore, we considered three sets of correlation values. In our initial scenario, we used a correlation of 0.9 for occupied subplots and 0.3 for unoccupied subplots. We also considered a high-correlation scenario, with a correlation of 0.9 for occupied subplots and 0.4 for unoccupied subplots, and a moderate-correlation scenario, with a correlation of 0.8 for occupied subplots and 0.3 for unoccupied subplots.

Effort

We considered efforts of 1,000, 2,000, 4,000, or 8,000 acres. For different levels of effort, we considered plots with 10, 20, or 40 1-acre subplots. Achieving a total survey effort with smaller plots will require more individual plots and therefore, more travel time and labor cost, especially because plots might not be near roads.

Survey Effort and Precision of Estimates

This protocol was designed to estimate occupied acres and provide information on the trade-off between survey effort and precision. It is ultimately the manager's responsibility to determine the level of effort to invest in a monitoring protocol, in relation to the level of precision needed to inform management decisions. This protocol therefore includes several alternative scenarios for estimating some parameters, giving the manager the option to reduce survey field effort at the cost of estimate precision.

In our initial simulations, we focused on the effect of plot size on the precision of estimates. We set occupancy to 0.75, detection to 0.4, and correlation to 0.9 (occupied subplots) and 0.3 (unoccupied subplots). Under these settings, 56.25% of all acres would be occupied. We then considered efforts of 1,000, 4,000, or 8,000 acres using plots containing 10, 20, or 40 1-acre subplots. We found that although total effort affected the precision of estimates, plot size had little effect (Table 1, Figure 1). We arbitrarily selected a target of estimating occupancy ±7.5% (i.e., 95% confidence interval of 48.75% – 63.75). Given the assumed parameter values, it would require surveying approximately 8,000 acres to achieve this level of precision (Figure 1, Scenarios 7-9).

We then performed additional simulations with different parameter values. With occupancy of 0.90, and correlations of 0.9 (occupied subplots) and 0.3 (unoccupied subplots), we expected 72% of all acres to be occupied. We considered both high (0.6) and low (0.4) detection probabilities. We considered efforts of 1,000, 4,000, or 8,000 acres using plots with 20 1-acre subplots. We then lowered the correlations to 0.8 and 0.4, retaining all other settings. In this case, we expected 54% of all acres to be occupied. With the lower level of detection probability, it was not possible to achieve the desired level of precision with the survey effort we considered (Table 2, Figure 2, Scenarios 10-12, 16-18). At the higher detection probability, the target precision was met between 4,000 and 8,000 acres of surveys (Figure 2, Scenarios 14-15, 20-21).

If actual parameter values differ from the values assumed here, precision will differ as well. Given that this survey protocol has not been applied to prairie dogs, it may be prudent to initiate a pilot survey to refine the method and generate estimates of key parameters, such as detection probability and correlation between subplots.

PROTOCOL

Prior to initiating fieldwork, the above procedures should be used to determine the number and location of plots, and the number of acres surveyed per route. Surveys should be conducted during seasons and times of day when prairie dogs are most active. In Arizona, GPD are most active from late March to mid-August, and from 7:00 to 10:00 and from 14:00 to 18:00 (AGFD unpublished data). Surveys should not be conducted if wind speed exceeds 23 mph, or during moderate to heavy rainfall. At the beginning of a survey, a surveyor will approach the first 1-acre subplot (without entering the plot) and stop 64 m away from the edge. To aid navigation, vantage points will be downloaded into GPS units prior to the survey. From this vantage point, the surveyor will observe the 1-acre subplot. The purpose of surveying from outside the plot is to reduce hiding behavior by the prairie dogs. The surveyor may use a sighting device to help delineate the 1-acre subplot (see Protocol Improvements, below). The surveyor will observe the plot for three minutes and record whether or not they observe prairie dogs. When the three minutes expire or after a prairie dog is observed, the surveyor should proceed to the next 1-acre subplot, maintaining a distance of 64 m. The surveyor should survey each 1-acre subplot until the entire plot is complete. If a subplot falls on a road, that subplot may be skipped.

In addition to prairie dog observations, surveyors should record their name, the location of each plot and subplot, the time, air temperature, cloud cover, and wind speed.

PROTOCOL IMPROVEMENTS

Visually estimating an acre can be difficult. If the surveyed sub-plots are not one acre in size, then the estimate of occupied acres will be biased. Furthermore, there is a tendency to expand observation areas to increase positive observations. Therefore, a sighting device may aid surveys. This device, possibly made of PVC, would include a chest-high pole and four short arms. The tip of each arm would function like a sight, and would indicate the corner of a plot. The sights would need to be calibrated against a measured one-acre plot prior to surveys. If the sight was consistently positioned, and the landscape were flat, it could accurately measure a plot. Although some measurement error would remain, it could increase objectivity about which observations should be included in a given sub-plot.

DATA ANALYSIS

Analysis could be completed in PRESENCE or in WinBugs. PRESENCE is menu-driven shareware that includes an option to analyze correlated-detection occupancy models. It returns estimates for occupancy of plots, correlation between sub-plots, and detection probability. However, the parameter of interest in this study is occupancy of sub-plots. Therefore, it will be necessary to derive an estimate of sub-plot occupancy from the other parameters. Presumably,

variance would be estimated for the derived parameter by applying the delta method to the variance-covariance matrix of parameter estimates. Alternatively, boot-strap resampling could be considered. The alternative software, WinBugs, is a Bayesian modeling language. A user would need to write an appropriate likelihood for analysis. Therefore, this approach requires more statistical and programming skill. However, Bayesian analysis simplifies estimation of derived parameters and especially the variance of derived parameters.

Future Review of Monitoring Protocols

Given that correlated-detection occupancy analysis is a new approach to prairie dog monitoring, and the population may change through time, results should be reviewed regularly and the protocol should be adjusted as necessary. We recommend that the protocol is reviewed after each of the first three years of implementation, and then at least every five years thereafter. The current recommended protocol for estimating occupied acres is based on rough estimates of occupancy of plots, detection of prairie dogs, and the level of spatial correlation. If data indicates that these estimates were inaccurate, it may be appropriate to revise the protocol. The protocol is intended to assist the Department in achieving management goals, such as assessing progress towards downlisting goals, and informing translocation decisions. If survey results are not adequate to inform current management decisions, or if new management scenarios arise, the protocol may be reassessed.

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APPENDIX C: GUNNISON'S PRAIRIE DOG DENSITY MAPPING PROTOCOL

PURPOSE

The purpose of this protocol is to estimate Gunnison's prairie dog (*Cynomys gunnisoni*) occupancy and density, which is used to estimate black-footed ferret (*Mustela nigripes*; hereafter BFF) family rating (FFR).

BACKGROUND

In addition to delineating the distribution of complexes and estimating occupied acreage, the density mapping method estimates density. The density mapping method converts the number of active burrows obtained from each transect to the number of active burrows per hectare, which is directly correlated to the number of prairie dogs per hectare (Biggins et al. 1993). The number of prairie dogs per hectare is used to predict the FFR. A BFF family is composed of one female, 3.3 kits, and 0.5 male.

The density mapping method spaces transects 500 m apart across the prairie dog colony. Similar to Biggins et al. (1993), the observer counts the number of active and inactive burrows. However in contrast to walking 1,000 m transects, the observer stops approximately every 250 m and records the number of active and inactive burrows within a radius of 1.5 m.

The Biggins et al. (1993) formula is used to calculate habitat data except for two variations: 1) the percent good habitat is calculated by the proportion of good habitat/total area on the density map, and 2) the active burrow density of good habitat is calculated in ArcVIEW as the mean active burrow density found in the areas with good habitat.

PROTOCOL

EQUIPMENT

1. GPS
2. Transect pole

FIELD PROTOCOL

1. Choose a starting point on the perimeter of the prairie dog colony.
    a. In even years, the last three UTMs numbers of the easting coordinate are a random number generated between 1 and 250. For example, if the random number is 175, add 500 and the other number would be 675.
    b. For odd years, the last three UTMs numbers of the easting coordinate are a random number is generated between 251 and 500. For example, if the random number is 375, add 500 and the other number would be 675.

   c. Transects are walked north/south using the last three UTMs numbers of the easting coordinate.
      i. Transects are spaced 500 meters apart.
      ii. A transect ends when there are no more active or inactive burrows visible.
         1. Scan ahead to determine whether the colony has ended or if there is a gap between burrows and the colony continues.
2. Use a transect pole to count all active and inactive burrows that fall within the three meters of the transect.
   a. Record active and inactive burrows every 250 meters along your transect.
      i. An active burrow will have fresh scat that is greenish, black or dark brown in color within 0.5 meters.
         1. Burrow entrances will be a minimum of 7 cm in diameter, the entrance will be open, and the end of the burrow cannot be seen.
      ii. An inactive burrow may have old scat that is dried and bleached white. A burrow is not considered active if there are only fresh diggings, tracks, or sightings.
      iii. The transect pole must be over at least half of the burrow entrance to be included.
3. The ending point will be at the end of the last transect that includes the opposing perimeter to your starting point.

FERRET FAMILY RATING

The FFR is calculated by following the instructions of Biggins et al. (1993, 2006). The FFR is used to determine whether prairie dog complexes are sufficient to support a BFF reintroduction. It is not an absolute measure of how many BFF can be expected to occupy a given complex.

1. Estimate the proportion of good habitat as the number of transects with at least 10.1 active burrows per acre divided by the total number of transects.
2. Estimate the area of good habitat by multiplying proportion of good habitat by colony size.
3. Calculate average density of occupied burrows for only good habitat. Because each transect covers 0.74 ha, at least eight occupied burrows must have been counted along each transect (10.1 occupied burrows/ha multiplied by 0.74 ha).
4. Convert the density of occupied burrows to density of prairie dogs (PD DEN).

$$\text{PD DEN} = (0.073 \times \text{active burrow density}) / 0.495$$

5. Estimate the number of prairie dogs on good habitat by multiplying the result of calculation number 2 by the result of calculation number 4.
6. Estimate the number of BFF family groups that the colony supports by dividing the result from calculation number 5 by 763. If the result is less than 272.5, the colony receives a rating of zero (0).
7. The rating for the complex is the sum of all colony ratings.

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APPENDIX D: GUNNISON'S PRAIRIE DOG POINT COUNT SURVEY PROTOCOL

PURPOSE

The purpose of this protocol is to determine Gunnison's prairie dog (*Cynomys gunnisoni*) population trends using a rapid assessment survey tool.

BACKGROUND

Occupancy, perimeter and density mapping protocols take considerable time to implement and even longer to analyze the data in order to assess the status prairie dog populations. Point Count Surveys can be implemented to quickly determine trends in prairie dog populations in order to inform protective management decisions. Point count surveys are a road based presence/absence survey conducted at the same location and at the same time annually to determine trends. In areas that show a sudden contraction of GPD occupancy range, the Department can investigate the causative agents and apply protective measures.

PROTOCOL

EQUIPMENT

1. GPS
2. Binoculars

FIELD PROTOCOL

1. Timing
    a. Conduct surveys during daylight hours preferable in the morning and evening.
    b. Do not conduct surveys during extreme weather conditions (i.e. winds greater than 25mph, heavy rains, etc.).
2. Delineate the road routes to maximize coverage of the study area but reduce redundancy.
    a. Routes should be spaced a minimum 0.25 miles.
3. Start the survey at the southwestern point of the study area.
4. From the vehicle, scan for prairie dogs. Presence is determined by visual confirmation of a prairie dog, not by vocalizations and/or scat.
    a. Scan for prairie dogs prior to recording data as the prairie dogs may go below ground quickly after arrival.
5. Survey the area for five minutes.
6. Data records:
    a. For each route, record
        i. Observer.
        ii. Date.
        iii. Route.

iv. Starting UTM (NAD83).
v. Direction of travel.
vi. Weather
vii. Start and stop times of the survey route.

b. For each point observation, record:
i. Prairie dog presence or absence.
ii. Location of the animal relative to the road.
iii. Additional notes.
1. Notes include; unsuitable habitat, other observed species, human or predator disturbance, etc.

c. Each hour record weather;
i. Temperature - In the shade at least one meter off the ground.
ii. Percent cloud cover.
iii. Average wind speed over 30 seconds.
iv. Precipitation and an estimate of the amount of precipitation i.e. sprinkles, downpour

7. Travel 0.5 miles (as determined by the truck odometer) to the next point.

APPENDIX E: DISEASE SURVEILLANCE PROTOCOLS FOR SYMPATRIC SPECIES

PURPOSE

This protocol provides guidance for disease sampling for the safe collection, handling, and storage of tissue, carcass, and blood samples from prairie dogs and sympatric rodents, rabbits, mammalian mesocarnivores (coyotes, foxes, and badgers) and fleas (burrow swabbing) within current and proposed black-footed ferret (*Mustela nigripes*; hereafter BFF) reintroduction sites.

BACKGROUND

BFF populations depend on prairie dogs for food and shelter, therefore populations naturally cycle together. One method for determining if disease is present within a Gunnison's prairie dog (*Cynomys gunnisoni*; hereafter GPD) population is to collect blood and tissues from sympatric species at random sites within the colony. In addition, sympatric mesocarnivores are exposed to pathogens through consumption of infected prey and an infestation with infected fleas and ticks. Disease surveillance in mesocarnivores can be used to detect canine distemper, plague, and tularemia, however, surveillance may incorporate additional diseases such as leptospirosis, or coccidioidomycosis or, through advanced molecular methods, currently uncharacterized pathogens. Mesocarnivores can be exposed to these pathogens and develop titer levels without succumbing to the disease. These samples can be collected in one of two ways; lethal collection of sympatric prey (prairie dogs, rabbits and rodents) and mesocarnivores (badgers, foxes, and coyotes) species for necropsy, or live trapping the same sympatric species, collecting blood and fleas.

Opportunistic disease surveillance in current BFF reintroduction sites will be conducted throughout the year. Surveillance in proposed sites prior to reintroduction will be systematic and preemptive. Additional surveillance will be conducted on MA when GPD and rodent population monitoring detects a significant increase in population density as a result of weather conditions favorable to forage production which could then precipitate a plague outbreak (Abbot and Rocke 2012). To conduct statistically valid surveillance for disease within a prairie dog complex, a significant number of GPDs, other small mammals, mesocarnivores, and burrows (fleas) need to be sampled from sites randomly distributed across the colony. After creating a density map of the colony, stratified sampling sites which represent different habitats and prairie dog densities should be randomly selected. Sampling should not exceed 1% of the existing GPD population if lethal collection is being used. The number of samples collected at each site will vary with GPD density and the number of sampling sites will depend upon the overall size in hectares of the colony.

Predator disease sampling is done using the whole body, head, swabs, tissues, and blood. Samples should be taken as close to the BFF reintroduction area as possible but can be up to six miles from the site for coyotes and four miles for badgers and foxes (Messick and Honocker 1981, Rosatte and Allen 2009, Kamler et al. 2003). The decision to perform lethal vs. live animal sampling will depend on BFF population trends, target population trends, signs observed, habitat

conditions, and urgency. Lethal collection followed by a complete necropsy allows for a broader spectrum of testing than the collection of blood and other biological samples and in the event of an epizootic, will be a necessary part of the investigation process. Alternatively, repeat sampling of live animals provides information regarding changes in exposure rates and residency. If shooting, non-lead ammunition will be used. Should a possible disease event be detected in any of the sympatric species on a BFF reintroduction site, active surveillance and response to a mortality event will be conducted according to the Significant Terrestrial Wildlife Disease Event Response Plan.

PROTOCOL

1. Equipment.
    a. Trapping equipment.
        i. Havahart traps for prairie dogs and mesocarnivores.
        ii. Sherman traps for small mammals.
        iii. Leghold traps for mesocarnivores.
        iv. Each trap should have its own number written or marked on the trap or flag.
        v. Bait - Oats, sweet feed, and/or peanut butter.
        vi. Stakes.
        vii. Tags.
        viii. GPS and/or flags.
        ix. Trap covers such as plywood or burlap.
    b. Standard processing equipment.
        i. Scale (1kg).
        ii. Linen bag.
        iii. Permanent markers.
        iv. Rulers or calipers.
        v. Latex gloves.
        vi. Leather handling gloves.
        vii. Canvas bag.
        viii. Tweezers.
        ix. Data sheets and clipboard.
        x. Camera.
    c. Blood collection equipment.
        i. Nobuto strips.
        ii. Magnetic clips.
        iii. Small envelopes or zip-lock bags.
        iv. 22 gage needles.
        v. Vacutainers or other blood collection tubes, capillary tubes, microtainer tubes.
        vi. 4.0-5.0 mm lancets.
        vii. Small electric razor.
        viii. Cotton balls or gauze.
        ix. Quick stop or styptic powder.

x. Hydration kit.
    1. Normosol.
    2. Lactated ringers.
    3. Syringes.
    4. Needles.

d. Anesthesia equipment.
    i. Isoflurane.
    ii. Anesthesia chamber.
    iii. Anesthetic drugs (typically telazol or ketamine and medetomidine, atipamezole).
    iv. Syringes and needles.
    v. Bleach.
    vi. Paper towels.

e. Flea collection equipment.
    i. White plastic tray.
    ii. Water.
    iii. Comb.
    iv. Forceps or tweezers.
    v. Vials or tubes.
    vi. Alcohol.
    vii. Swab tool.
    viii. Flagging.
    ix. Plastic bags.
    x. Insect repellent (DEET).

f. Mesocarnivore sample collection equipment
    i. Data sheets and clipboard.
    ii. Necropsy kit.
        1. Knife.
        2. Scalpel.
        3. Scissors.
        4. Forceps.
        5. Formalin jars.
        6. Whirl packs.
    iii. Nobuto strips.
    iv. Magnetic clips.
    v. Small envelopes or zip-lock bags.
    vi. 22 gage needles.
    vii. Vacutainers or other blood collection tubes, capillary tubes, microtainer tubes.
    viii. 4.0-5.0 mm lancets.
    ix. Swabs, glycerol/TSB, cryovials.
    x. Small electric razor.
    xi. Cotton balls or gauze.
    xii. Quick stop or styptic powder.
    xiii. Personal protective equipment.
        1. Gloves.

2. Masks.
3. Aprons.
4. Goggles.

xiv. Cleaning supplies.
1. Garbage bags.
2. Disinfectant.

xv. Camera.
xvi. Sharps container.
xvii. Centrifuge if spinning blood.
xviii. Cooler and gel ice packs or frozen water bottles.
xix. Shipping supplies

SMALL MAMMALS

1. Objectives – in order of importance.
   a. Collection of fleas and blood from Northern grasshopper mice
   b. Collection of fleas and blood from additional rodents and sciurids other than GPD
   c. Collection of fleas and blood from GPD
2. Target sample size:
   a. Sample 5% of perimeter (if perimeter is 75 miles then 4 arrays are needed)
   b. Trap arrays will be randomly located around the perimeter of the MA and in habitat rated as good BFF habitat (i.e. 10.1 occupied burrows/acre) to maximize the number of animals sampled.
   c. Trap arrays will consist of 100 live traps (75 Sherman for small rodents and 25 Havahart for GPD) spaced 15m apart on a 3 by 25 trap grid for the Shermans and Havaharts placed at active burrow entrances (Kraft and Stapp 2013).
3. Anesthesia of rodents.
   a. Sedate animal using isoflurane for flea collection in known or suspect plague areas.
      i. Saturate cotton ball with isoflurane and put in stainless steel mesh tea ball.
      ii. Place prairie dog in chamber with saturated cotton ball (sedation typically occurs within 1-2 minutes, duration is 1-5 minutes).
      iii. Monitor animal's breathing to determine sedation level.
      iv. Overexposure to isoflurane will result in mortality.
   b. Check isoflurane chamber for fleas that may have fallen off after sedation.
   c. Clean chamber and all equipment between use with a 1:10 dilution of household bleach solution, wait 10 minutes and wipe with a wet paper towel to rinse.
   d. Allow animal to fully recover from anesthesia before release at capture location.
4. Blood collection.
   a. Nobuto Strips (2) are used to collect blood to test plague.
   b. Blood collected with syringe or by cutting toe nail (toe nail collection preferred).
   c. Use nail clipper or cat claw scissors and cut a 45º angle just below nail bed.
   d. Collect blood on strip as it drips from the nail.
      i. Apply blood to both sides of long narrow strip (see Figure 1).
   e. Allow nobuto strips to dry before storing in envelope DNA envelope.
   f. Label envelope.

i. Site name.
ii. Trap number.
iii. UTMs and datum.
iv. Date.

g. Apply pressure to the nail to stop bleeding.
    i. If bleeding does not stop, apply styptic powder, silver nitrate, or super glue.
h. Nobuto strips may be stored at room temperature or frozen in an archive.
    i. Do not allow the strips to get hot.
i. Send Nobuto strips to Center for Disease Control (CDC), Bacterial Disease Branch.

5. Small Mammal Carcass Collection.
    a. Trapping and anesthesia with isoflurane or telazol followed by euthanasia via cervical dislocation, pithing, or intracardiac euthanasia solution is preferred.
    b. Shooting using a small caliber non-fragmenting, non-lead bullet targeting the head to preserve as much of the carcass as possible.
    c. Salvage found animals found dead when the carcass is intact (not scavenged), eyes are not desiccated, and maggots are not present.
    d. Double bag carcass in labeled ziplock.
        i. Site name.
        ii. UTMs and datum.
        iii. Collector's name, affiliation, contact information.
        iv. Type of animal.
        v. Date.
    e. Ship carcass to Arizona Game and Fish Department Wildlife Health Program Supervisor for necropsy.
6. Flea Collection.
    a. From live animals.
        i. Trap animals at each sampling location for collection.
        ii. If necessary, sedate animal using isoflurane for flea collection.

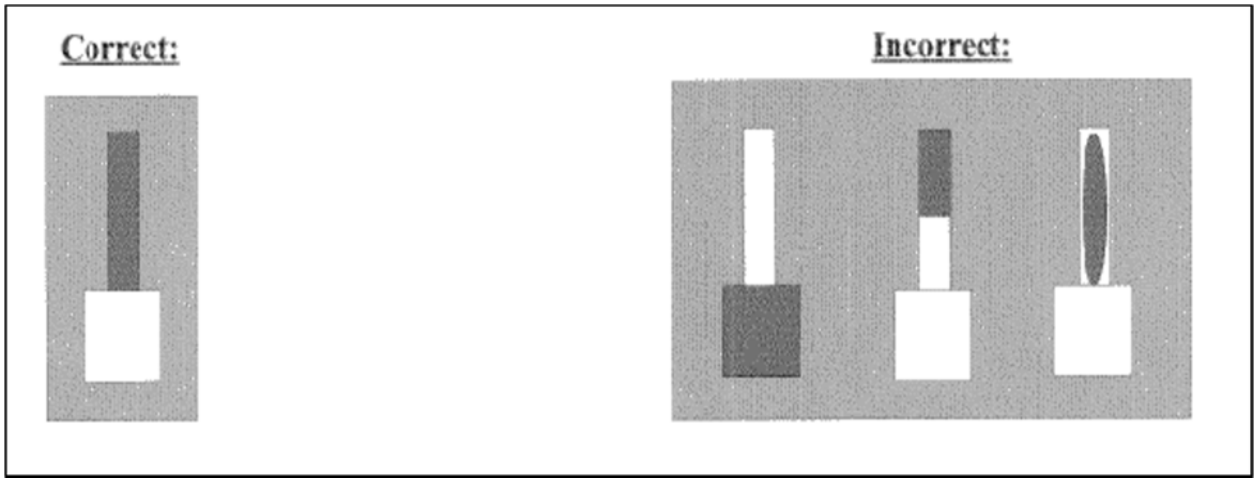

Figure 1. Use of Nobuto strips for blood collection, cover narrow end completely with blood and allow to air dry before placing in a paper envelope. Keep in a controlled environment, may be frozen but should not be refrigerated (allows mold to grow).

iii. Hold animal over tray of shallow water and comb body thoroughly to allow fleas to fall into the water for easier collection.
iv. Check isoflurane chamber for fleas that may have fallen off after sedation.
v. Collect fleas and store in vials with alcohol.
vi. Label vial
    1. Site name.
    2. UTMs and datum.
    3. Date.
vii. Chill or freeze vials as quickly as possible.
viii. Send flea samples to Northern Arizona University, Center for Microbial Genetics and Genomics.

b. Burrow swabs.
i. Attach flag to swab tool.
ii. Force flag in to burrow as far as possible.
iii. Slowly withdraw the flag.
iv. Quickly place the flag in the plastic bag and seal it.
v. Repeat i-iv for each burrow.
vi. Record the number of fleas collected for the burrow and the number of burrows swabbed.
vii. Keep the samples cool.
viii. Freeze the bags overnight.
ix. Remove the fleas from the flagging by placing it in a white enamel pan and collecting the fleas with forceps.
x. Place the fleas in saline in leak proof vials.

MESOCARNIVORES

1. Objectives and sample size
    a. Sufficient to detect seroprevalence of 5% or greater with a 95% confidence based on the estimated population size of coyotes and other predators.
    b. Identify with 95% confidence interval increases in seroprevalence above 5%.
    c. Estimate abundance of predators in MA
    d. Using table below, determine number of samples to collect within 4 miles of MA

Population size	Sample size
20	19
30	26
40	31
50	35
60	38
70	40
80	42
90	43
100	45
140	48
200	51

From Thrusfield 2007

2. Specimen acquisition.
    a. Live trapping.

i. Set traps in evening near identified fox or badger burrows or along game trails according to standard practices.
ii. Check traps in morning.
iii. Sedate animals with an intramuscular injection of prescribed drugs.
iv. Tag animal before releasing with a PIT tag between the shoulders and an eartag.
v. Reverse sedation and recover animal after collecting samples.
vi. Release at capture location when fully recovered.

b. Lethal acquisition.
i. Trap and euthanize with CO_2 or euthanasia drug or shoot with non-lead ammunition in neck or head.
ii. Carcass may be double-bagged in garbage bag and labeled.
1. Site name.
2. UTMs and datum.
3. Collector's name, affiliation and contact information.
4. Type of animal,
5. Date.
iii. Taken on ice to a central processing site,

3. Collect samples, processing station.
a. Always wear gloves when handling mesocarnivores.
b. Locations for each predator collected should be recorded using UTMs in NAD 83. Sample numbering system includes a two-digit site identifier, two-digit year, and specimen number for that year, example: AV16-0001for first specimen collected in the Aubrey Valley 2016. Sample number should be accurately recorded on all sample containers and the data sheet.
c. Blood, live animal.
i. Nobuto strip from trimmed nail as above.
ii. With syringe and needle from jugular, cephalic, lateral saphenous or femoral vein (dispose of needle in sharps container).
iii. Place blood in vacutainer tube.
iv. Keep cool, do not freeze.
v. See below for blood processing.
d. Blood, carcass.
i. Collect two Nobuto samples.
ii. Blood sample should be taken immediately after animal is shot, if possible. Do not sample specimen if dead longer than eight hours.
iii. Cut chest cavity with scalpel.
iv. Take approximately 10-ccs blood sample from heart using 10-cc syringe and 12-gauge needle.
v. Transfer blood to two vacutainer blood tubes (5-cc in each tube) labeled with the sample id through rubber cap.
vi. Promptly dispose of needle in sharps container after use.
vii. Keep cool, do not freeze.
viii. See below for blood processing.
e. Feces.
i. With fecal loop or swab.

ii. Place in media, whirl pack, or tube.
iii. Keep cool, do not freeze

f. Oral pharyngeal swab, live animal.
i. Use two pieces of gauze, twine, or a mouth gag to hold open mouth.
ii. Swab the back of the throat, soft palate and tonsils.
iii. Place swab in glycerol/TSB or cryovial.
iv. Freeze.

g. Respiratory tract swabs, carcass.
i. Open trachea longitudinally from middle of neck/chest and cut down through the airways to lungs; rub swabs down trachea into smaller airways.
ii. One swab in TSB/glycerin medium.
iii. One swab in virus medium (break off extra length of stick to secure vial shut).
iv. Label vials with respective specimen number.
v. Freeze tubes.

4. Collect samples, field
a. Always wear gloves when handling mesocarnivores.
b. Whole head.
i. Remove head as close to the shoulder as possible if carcass is in good condition.
i. Place in double plastic bags labeled with specimen number and freeze.
ii. Keep as much of brainstem intact as possible.
iii. Submit the head to Wildlife Health Program.

b. Tissue sample collection.
i. Head or brainstem, heart, lung, spleen, liver, kidney, bladder, and stomach.
1. Collection size.
a. Approximately the size of a quarter and ¼ inch thick or less.
2. Collect at least four tissues, collect one to four samples per organ.
3. Place tissue samples in container with 10% formalin, label with specimen number.
4. Always replace scalpel blade between animals to prevent cross contamination.
5. Tissues should not take up more than 10% of the volume of formalin in the jar.
6. Label jar with date, specimen id, and location.
7. Tighten lid and seal with waterproof tape, or paraffin film.
8. Double bag the head and submit to the Wildlife Health Program.
9. Submit to Washington Animal Disease Diagnostic Laboratory and cc the Wildlife Health Program.

ii. Intestines should also be collected.
1. 2-3 inches of small intestine.
2. Place in ziplock or whirl pack labeled with date, specimen id, and location.

3. Freeze.
4. Submit to Predator/Furbearer biologist for testing.

c. Specimen collection.
   i. Use checklist on Data Collection Form as samples are taken.
   ii. Collector is responsible for carcass disposal.

d. Blood Sample Processing.
   i. Allow blood to clot in tube (approximately 30 minutes).
   ii. Immediately place samples on ice in a cooler until they can be properly centrifuged and stored.
   iii. DO not freeze samples.
   iv. Spinning and shipping blood.
      1. Spin blood for 10 minutes at 3500 rpm.
      2. Pour or pipette serum into small vials labeled with specimen number and freeze.
      3. Place serum vials in one whirl-pak bag.
      4. Keep blood, and/or serum cool when shipping; always use ice packs.

SAMPLE SUBMISSION

1. Contact the appropriate individual and advise of shipment.
2. Verify shipping method and sample submission form.
   a. Washington Animal Disease Diagnostic Lab – general and multiple animal.
   b. Identify tests requested.
   c. Can use a spreadsheet for some testing labs and the Wildlife Health Program.
   d. Be aware that many testing labs assign their own sample identification, will need to track with program sample identification.
3. Send a copy of the forms to the Wildlife Health program.
4. Shipping addresses and contact information.

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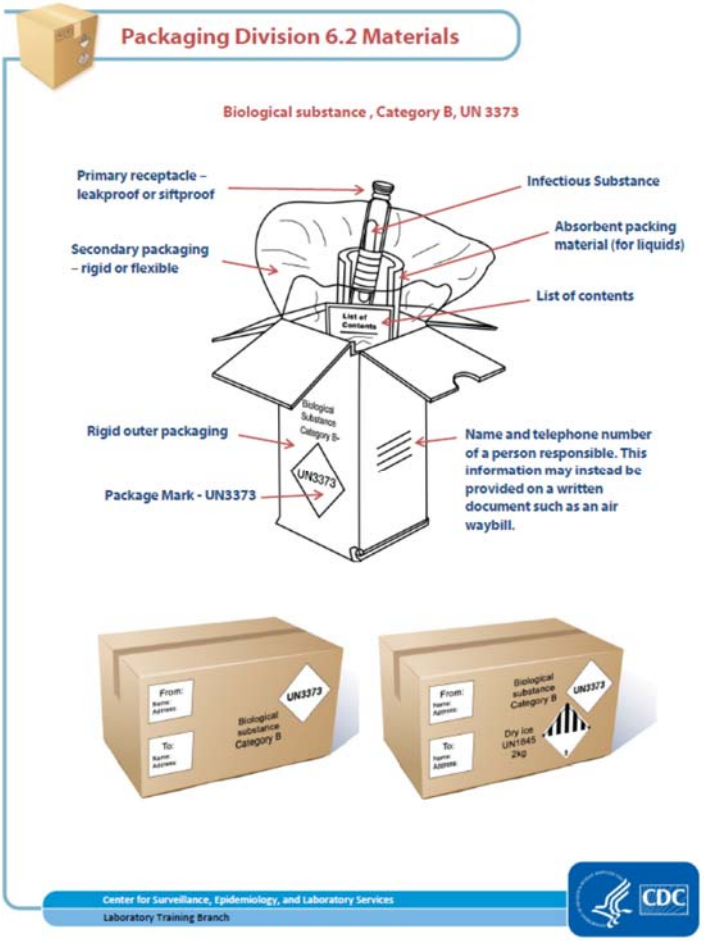

Figure 2. Sample packing instructions.

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APPENDIX F: BURROW DUSTING PROTOCOL

PURPOSE

This protocol provides guidance on application of insecticide on prairie dog colonies to control fleas and reduce exposure and mortality to plague epizootics.

BACKGROUND

The Modern Plague is a bacterial disease (*Yersinia pestis*). In the late 1800s, the disease was spread by fleas through rat-infested ships from China. The first plague outbreak in the United States was confirmed in Hawaii in 1899 and San Francisco in 1900-1904 (Chase 2003). The first continental epizootic was confirmed in California ground squirrels in 1908; however, the disease soon spread to other North American mammals.

Prairie dogs are extremely susceptible to plague, which has caused large mortality events. The application of topical insecticide has been an effective means of reducing flea loads and plague-caused mortality within a colony. The most common type of insecticide used for plague outbreaks is Deltamethrin or Delta Dust, which has a permethrin base. Deltamethrin is a powder; thus the procedure to apply the insecticide is called "dusting." However, dusting is logistically expensive and only locally effective, therefore its use must be evaluated for effectiveness.

PROTOCOL

EQUIPMENT

1. Pesticide Applicators License
2. Deltamethrin (DeltaDust).
3. Applicators.
4. Respirator masks.
5. Protective Clothing.
6. Markers (flags or spray paint)
7. GPS

Field Protocol

1. Identify the treatment area.
    a. Treat only when prairie dogs are active (April-October).
    b. Deltamethrin is toxic to aquatic wildlife and should not be applied within twenty feet of any riparian areas including but not limited to stock ponds, streams and rivers, and lakes, and reservoirs.

   c. If the treatment area is logistically too large (e.g. 10,000 acres), focus on the highest densities of prairie dogs or any black-footed ferret (*Mustela nigripes*) locations.
   d. The number of personnel required depends on the size of area to be dusted.
2. Start at the colonies perimeter and apply treatment to every burrow (prairie dog and other rodent burrows).
   a. Applicators should be equipped with a nozzle that directs the dust into the burrow and limits the dust from being carried away by the wind.
   b. Personnel should direct the dust as deep into the burrow cavity as possible.
      i. Coat the inside of the burrow entrance on all sides, applying approximately 4-6 oz of insecticide to each burrow. This can be calibrated for distribution or timed depending on the applicator.
   c. Personnel should systematically apply treatments across the colony, regardless of animal use.
      i. Use markers to prevent double dusting.
3. After treatment, map the area with a GPS.
   a. Document the date, acreage treated, and the number of burrows dusted.
      i. If BFF are within the treatment area, report the treatment to the USFWS.

LITERATURE CITED

Chase, M. 2003. The Barbary Plague: the black death in Victorian San Francisco. Random House, New York, USA.

APPENDIX G: BLACK-FOOTED FERRET MONITORING PROTOCOL

PURPOSE

This protocol includes methods for estimating the following:

1. Absolute abundance of adult black-footed ferrets (*Mustela nigripes*; hereafter BFF) across a large landscape (such as the Aubrey Valley). The protocol is designed to be feasible to conduct annually, but managers may choose to estimate this parameter less frequently, and to use an index of abundance (Objective 2) to estimate a population trend in alternate years. This protocol is designed to be transferrable to other parts of the state.
2. An index of abundance of adult BFF across a large landscape (such as the Aubrey Valley). This index could be used to track trends in population size, given certain assumptions hold, but the index does not provide an estimate of absolute abundance.
3. Annual BFF survival for each age-sex class. This estimate requires at least two years of data.
4. Annual BFF reproduction, based on offspring recruitment to a specific age.

BACKGROUND

Spotlight and trapping surveys of BFF have been the primary techniques for locating animals and estimating the minimum number alive. Spotlighting is an efficient approach for locating BFF, relative to such alternatives as track plates, scent dogs, scent stations, camera traps, and snow tracking (Biggins et al. 2006). Capturing BFF in traps allows individual marking, as well as examination and vaccination. Typically, the number of unique animals encountered during surveys is reported as a minimum number alive (MNA; Biggins et al. 2006). However, the MNA is likely to underestimate true abundance due to imperfect detection of animals and incomplete spatial coverage (sampling) of field sites. Likewise, estimates of survival are underestimated using methods that require or assume perfect detection. Furthermore, if detection probability changes through time, the MNA will also fail as an index of abundance used to estimate trends. It has been argued that in areas inhabited by black-tailed prairie dogs (*Cynomys ludovicianus*; hereafter BTPD), the twin problems of partial spatial coverage and imperfect detection are not severe (Biggins et al. 2006). However, BFF in Arizona primarily occupy Gunnison's prairie dog colonies (*Cynomys gunnisoni*; hereafter GPD), which are larger, with more vegetation, than BTPD colonies (USFWS 2013). Therefore, imperfect detection and partial spatial coverage are more likely to be important issues.

The approach presented in this protocol uses spotlight and trapping surveys as a field technique, but applies it in the context of spatially explicit mark-recapture (SECR) methods on sample units selected with generalized random tessellation sampling (GRTS). The purpose of this approach is to account for imperfect detection so that state variables such as abundance may be estimated, and to account for incomplete spatial coverage to allow inference from sampled areas to the entire study area. Accounting for imperfect detection requires that some individually marked animals be captured multiple times and that data on survey effort be recorded. Model-based inference requires that sampled locations are selected using a probability-based method, and that

sampled locations represent a range of any features that could affect the density or detection of animals. For example, if animal density varies with distance to road, sampled locations should occur at a range of distances from roads to improve estimates of the relationship between distance to roads and animal density. The GRTS approach selects sample units by applying a systematic random sampling to a hierarchical spatial tessellation of sample units. The result is a spatially representative, scalable selection of sample units.

SECR differs from traditional capture-recapture methods in that capture data are augmented with data on the locations of captures. While traditional capture-recapture methods generate abundance estimates, they are not associated with a well-defined area. In contrast, SECR includes a formal mechanism to estimate the area used by animals, enabling density estimates and extrapolation to larger areas. This is a key consideration for a wide-ranging species such as the BFF. Furthermore, SECR allows modeling of location-induced detection heterogeneity, a potential source of bias in traditional capture-recapture. Furthermore, analysis of multiple years of recapture data with a SECR model enables estimates of survival and recruitment.

Recognizing that monitoring budgets can change, this protocol also describes strategies for surveying BFF with a reduced budget. These strategies include conducting SECR surveys with reduced effort, counting BFF without capturing them, and not surveying BFF. Reduced-effort SECR surveys could be used to estimate abundance with reduced precision. Counts of BFF could serve as an index to population changes, under the assumption that detection probability is relatively constant among years. As such, counts would not estimate abundance, and are vulnerable to bias, but could be a signal of precipitous population changes. No surveys would provide no information, but might conserve resources for future thorough surveys.

PROTOCOL

SELECTION OF AREAS TO SURVEY

Selecting areas to survey requires the following steps, which are described in detail below.

1. Define the population of interest.
2. Define a feasible sampling frame.
3. Define strata.
4. Delineate sample units.
5. Weight sample units for selection.
6. Select sample units.

Population of Interest

Prior to designing a survey, it is necessary to define the population of interest. This is the collection of individuals about which information is desired. This might be a genetically or physically isolated set of BFF (a biological population), or it might be all BFF within a political boundary. Alternatively, a population might be defined by a management boundary. Once the population of interest is identified, surveys should be designed to gain information about this population (see Box 1). If the population is delineated by artificial boundaries (e.g., a

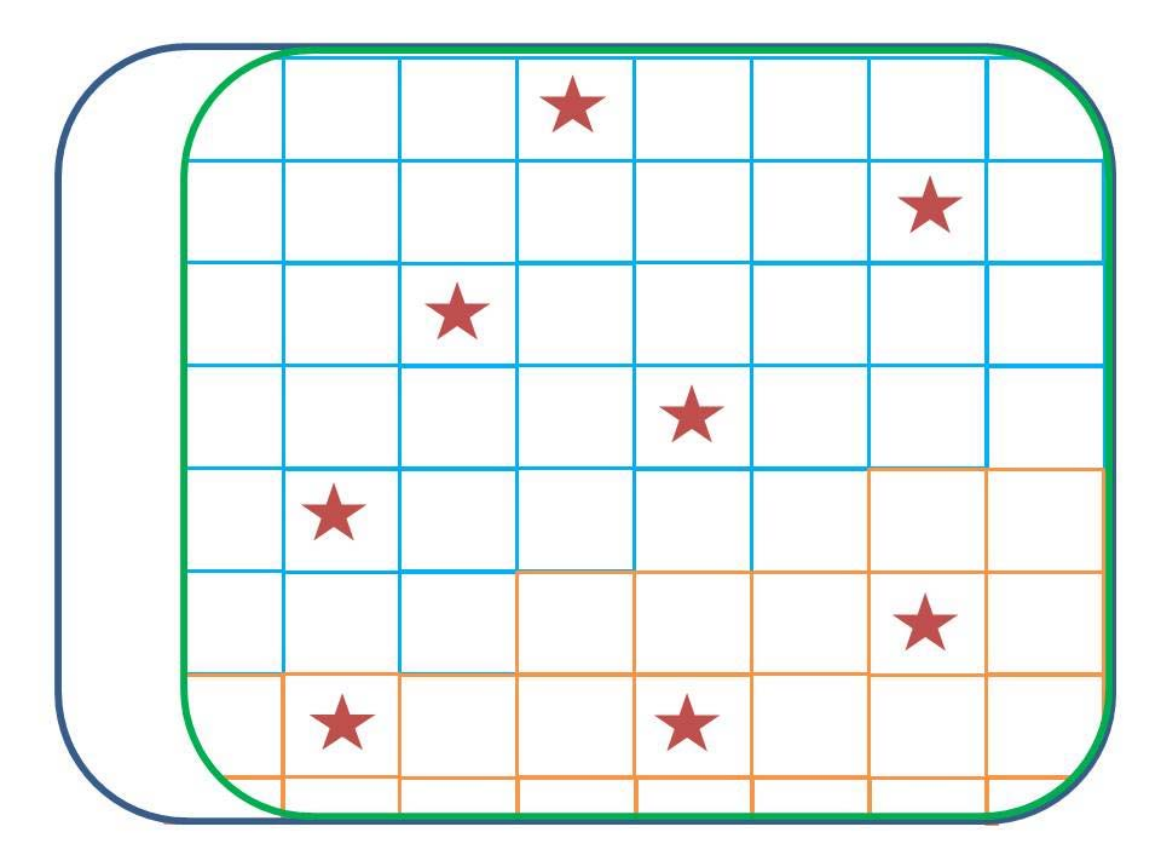
management area or political boundary) it is important to only make inferences to this subset of the biological population.

Sampling Frame

A sampling frame divides the population of interest into a comprehensive list of identifiable and observable elements. In the case of BFF, no comprehensive list of individuals is available. Instead, the sampling frame could consist of a comprehensive list of areas that could be surveyed for BFF. A truly comprehensive sampling frame should include all members of the population of interest. However, if population of interest covers a particularly large area, or is partially on inaccessible land, the sampling frame may cover only a portion of the population. In this case, the strongest inference will be limited to the sampling frame, not the entire population. If the population has the potential to move during the course of long-term monitoring, it is advisable to expand the sampling frame to cover potential habitat from the beginning of the monitoring effort.

Box 1: Sampling Concepts

Population: All individuals of research interest. Contained in dark blue shape.
Sampling Frame: Population organized into a comprehensive list. Contained in green shape. A portion of this population is excluded from the sampling frame.
Sample Units: Individual items within the sampling frame that could be selected for surveys. Each orange and light blue shape is a sample unit. Sum of all sample units equals the sampling frame.
Strata: Distinctive portions (e.g., habitat types) of sampling frame that may be treated differently during sampling. Orange shapes and light blue shapes are different strata.
Sample: The sample units that have been randomly selected for surveys. Shapes containing red stars were selected to comprise the sample.



Define Strata

It may be efficient to define multiple strata within a sampling frame, if habitat characteristics might influence BFF densities or labor costs. For example, a sampling frame could be divided into a road-side stratum and an off-road stratum. The roadside stratum could include paved roads, dirt roads, and passable two-tracks. Roadside surveys allow greater search efficiency and accommodate volunteer surveyors (useful if there is a need for outreach and matching funds). In this case, an off-road stratum is necessary to ensure that the sampling frame is comprehensive. Alternatively, strata might include grazed grasslands and un-grazed grasslands, if it is thought that BFF density differs among strata. Again, these strata should cover the entire sampling frame.

Delineate Sample Units

At large study sites, it is not possible to survey the entire sampling frame. In this case, the sampling frame should be divided into smaller sample units, some of which will be selected for surveys. Recent Arizona Game and Fish Department (Department) BFF surveys have used road-side sample units (called "routes") that are approximately 4 km (2.5 miles) long, while another survey used roadside units that averaged 243 ha (Grenier et al. 2009). Recent Department surveys do not have a defined width for roadside units. However, a review of Department BFF surveys from 2005 to 2016 determined that the number of detections at 200-250 m from roads was 12% of the detections at 0-50 m from roads, which is consistent with a recommendation to limit transect width when detections fall to 15% of the maximum (Buckland et al. 2001). Excluding captures beyond 250 m might exclude 8% of captures, but simplifies surveys and analysis. Therefore, roadside units could be defined to be 4 km long and 500 m wide (i.e., 250 m on each side of the road). Such units would be 200 ha. Roadside sample units do not need to be uniform in length, but if not, length should be recorded.

The off-road stratum should also be divided into sample units, so that the sampling frame is comprehensively covered. The size of off-road units can be selected for practical efficiency and tailored to the study area. For example, larger sample units would be appropriate at study areas that allow off-road vehicles, while smaller sample units would be appropriate where sampling is done on foot. Very small units waste time traveling among units. Very large units cannot be surveyed in a single night and reduce the spatial replication of samples. A previous mark-recapture survey for BFF that surveyed off-road units by foot delineated off-road units that were half the size of road-side units (Grenier et al. 2009). In that study, road-side units were partially surveyed by foot. If Department road-side units are surveyed entirely from vehicles, off-road units should be less than half the size of road-side units. Therefore, off-road units of 1.5 km by 500 m could be a reasonable size (75 ha), with north-south orientation and easting coordinates ending in 000 or 500. Off-road units that are truncated by intersection with roadside units or the edge of the study area will be smaller. To avoid the transportation costs associated with very small off-road units, units with a transect length less than 500 m or an area less than 25 ha could be excluded from the subsequent random selection under the assumption that inference would be minimally affected by excluding a few small units.

Weight Sample Units

It is not necessary, and sometimes inefficient, to sample strata in proportion to their availability (Skalski 1994). For example, if labor costs are higher in one stratum, reducing the probability of selecting units from that stratum will allow more total surveys and improve estimates. Given that

off-road units are smaller and require more transit time, selection could be weighted so that road-side units are selected at a greater proportion than their availability. To quantify the availability of units, the number of road-side and off-road units in the study area should be tallied. To account for size differences, the ratio of the mean size of road-side and off-road units should be calculated. To account for cost differences, the ratio of the mean labor cost of road-side and off-road units should also be calculated. The number of sample units should be determined using methods in "Survey Effort", below. From this information, simple simulations can calculate the appropriate ratio of road-side to off-road units. For example, if there are 50 road-side units and 500 off-road units, the size ratio is 4:1, the labor cost is 1:2, and we want to survey 30 units, then we should select 10 road-side units and 20 off-road units. Code for simulations is given in "Supplementary Material."

Select Sample Units

To draw conclusions about the population of interest, it is necessary that all sample units of sufficient size within the sampling frame have a known and non-zero probability of being selected for sampling. In contrast, in convenience sampling (such as when sampling only along roads), some sampling units (such as those away from roads) will have zero probability of being selected. While convenience sampling can be attractive because it can reduce costs and increase sample sizes, it is vulnerable to bias, samples cannot be generalized to the population of interest, and it is not possible to estimate variances. For these reasons, probability-based sampling is preferred.

Generalized random tessellation sampling (GRTS) is an effective tool for selecting a probabilistic sample from a sampling frame (Stevens and Olsen 2004). GRTS selects sample units by applying systematic random sampling to a hierarchical spatial tessellation of sample units. This approach generates better spatial balance than simple random samples, which reduces sampling error and improves model-based inferences. GRTS also provides more accurate variance estimates than systematic sampling. Another advantage of GRTS sampling is that it is scalable, so that if survey effort increases or decreases through time, the sample size can be adjusted without losing spatial balance.

Using GRTS in Program R requires a shapefile that describes the sample units (including location, strata, and weighting), and familiarity with R package 'spsurvey'. Properly specified, the 'grts' function will return a list of sample units to be surveyed. To make the sample scalable, R can be instructed to generate an ordered list of all sample units. In this case, if survey resources increase or decrease, the first *n* sample units can be selected, and the sample will remain random and spatially balanced. The number of sample units should be fixed before surveys begin. See "Field Protocol" below for guidance on how to deal with changes in survey resources (e.g., volunteer hours) that occur during the field season.

Existing Data and Simulations Used to Inform Development of the Protocol

The optimal survey design depends on the characteristics of the population under study. For example, different protocols will be optimal for high density and low density populations. Therefore, information about the population under study can help improve survey design. For SECR models, the most important parameters are population density, animal movements,

detection probability, and survey effort. Therefore, we obtained information on these parameters from the scientific literature and from previous BFF surveys conducted by the Department. We then used these parameters in a simulation study to inform survey design. Our simulations allowed us to examine resulting accuracy, bias, and precision of various sampling designs.

Density

The Recovery Plan (USFWS 2013) states that GPD habitat can support 0.03 ferrets/ha (p. 73; although the cited study does not seem to support this claim). However, suboptimal site conditions may reduce density to 0.01 ferrets/ha (USFWS 2013). A mark-recapture study in Wyoming estimated density at 0.027 ferrets/ha in white-tailed prairie dog (*Cynomys leucurus*) habitat (Grenier et al. 2009). Density in GPD colonies may differ, although the Recovery Plan expects density to be similar (USFWS 2013). A preliminary SECR analysis using data from the Department's BFF surveys conducted in Aubrey Valley estimated density at 0.027 ferrets/ha in 2013 and 0.015 ferrets/ha in 2015. However, because the Department intentionally selected survey plots that were anticipated to contain above-average densities of BFF, these estimates might over-estimate density. Therefore, we used densities of 0.005, 0.010, and 0.015 ferrets/ha to design surveys. At a site with 19,425 ha (48,000 acres) of habitat (approximately the size of the Aubrey Valley Management Boundary), these densities correspond to total abundances of 97, 194, and 291 BFF. The simulations also included non-homogenous densities, so that density varied sinusoidally around the mean density, to replicate the spatial variation in density found in natural populations.

Movements

The home range of BFF in BTPD habitat is reported to be 60 ha for females and 130 ha for males (Jachowski et al. 2010, Livieri and Anderson 2012). However, home ranges may be larger in Arizona, where BFF occupy GPD colonies, which are less dense. A preliminary SECR analysis using data from the Department's BFF surveys conducted in Aubrey Valley estimated a Gaussian scale parameter of 410 m in 2013 and 265 m in 2015. These values imply circular home ranges of 315 and 135 ha, respectively. Therefore, we used a home range of 250 ha (360 m Gaussian scale parameter) to examine simulated survey designs.

Detection

A mark-recapture survey for BFF in white-tailed prairie dog colonies estimated nightly detection probability at 0.52 (Grenier et al. 2009). In that survey, BFF were spotlighted in a combination of vehicle and foot surveys. Captured animals were dye-marked, so that animals could be visually "recaptured." Recapture rate was estimated at 0.45, suggesting a weak "trap-shy" effect. Another study in BTPD habitat found that nearly 40% of all BFF encountered during a survey were encountered on the first night, yielding a rough estimate of a 0.40 nightly detection probability (Biggins et al. 2006). A similar approach, comparing BFF found in the first night to total BFF found, suggested a nightly detection probability of approximately 0.32 for BFF in a white-tailed prairie dog colony (Forrest et al. 1988). Given the presence of more vegetation in GPD colonies, detection may be lower in Arizona. A preliminary analysis of the Department's BFF surveys in Aubrey Valley estimated detection probability at 0.16 in 2013 and 0.17 in 2015. The lower detection rate in Aubrey Valley is consistent with a previous comparison of BFF surveys that reported relatively low sighting rates in Aubrey Valley (Biggins et al. 2006). It seems reasonable that detection might differ in road-side units and off-road units, but a previous study did not

report any difference (Grenier et al. 2009), so no difference was allowed in the simulations. Given these data, we used a constant detection probability of 0.17 to design surveys.

Effort

A mark-recapture survey of BFF sampled 24 plots totaling 4,794 ha, with an average of 200 ha/plot (Grenier et al. 2009). Each plot was surveyed on three nights, which required 558 person-hours of effort. This implies each plot was surveyed for eight hours per night by a single person. Data on BFF survey effort are available for Aubrey Valley from 2009 to 2015 in the form of route-nights, or the sum of routes surveyed each night. Routes have been surveyed by multiple observers driving on paved roads, dirt roads, or two-tracks and searching with a spotlight. Considering only surveys from September and October and counting all partial route-nights as 0.5 route-nights, route-nights per year have been 30, 46, 58, 54, 69.5, 72.5, and 83. A previous study used 72 route-nights of effort (Grenier et al. 2009). Assuming a route length of 4 km (2.5 miles) and a width of 250 m (on each side of the road), each route would total 200 ha. However, route size has declined over time, so the change in route-nights overstates the increase in effort. Given the recent survey effort, we considered potential survey efforts of 90, 120, or 150 route-nights. A given effort can be allocated to increase the number of routes surveyed, or to increase the number of nights per route. We considered surveys with 3, 5, or 7 nights per route, with the number of routes determined by the total effort and the number of nights. For example, 90 route-nights and five nights per route yields 18 routes, while 150 route-nights and three nights per route yields 50 routes.

SURVEY EFFORT AND PRECISION OF ESTIMATES

This protocol was designed to estimate parameters identified in the objectives with reasonable precision while conserving resources (particularly personnel time). It is ultimately the manager's responsibility to determine the level of effort to invest in a monitoring protocol, in relation to the level of precision needed to inform management decisions. For example, it may be necessary to track population abundance more closely (with high precision) when a population is small, while it may be acceptable to estimate abundance with less precision when the population is large. This protocol therefore includes several alternative scenarios for estimating some parameters, giving the manager the option to reduce survey field effort at the cost of estimate precision.

We explored the relationship between survey effort and precision of estimates through a simulation study. We considered 27 scenarios developed from potential parameter values identified in "Existing Data and Simulations Used to Inform Development of the Protocol," above (Table 1). These 27 scenarios represented three densities (0.005, 0.10, 0.015), three survey efforts (90, 120, 150), and three study designs (3, 5, 7 nights per route) for dividing effort across sample units. The density of BFF is not under direct control of managers, but different levels were considered to account for uncertainty in the true density of BFFs. The Department can control survey effort. Increased survey effort will always improve estimates, but must be balanced against available resources. The primary purpose of the simulations is to select the most efficient study design, i.e., the number of nights per route and the number of routes to survey. For each scenario, we simulated 20 data sets and evaluated root mean square error (RMSE) of simulation results. RMSE is a measure of accuracy that accounts for bias and precision. Because RMSE can be difficult to interpret, we also report lower and upper confidence limits obtained

from the simulations. If parameter values in subsequent field surveys differ from those in the simulations, then bias and precision will differ as well. Bias and precision are particularly sensitive to the level and variation in detection probability, but are also affected by the level and variation in density and movement patterns.

Due to the low number of simulations per scenario, simulation results were somewhat ambiguous (Table 1). Nonetheless, it was apparent that surveying each route for only three nights was not optimal because RMSE was always higher, and because a few simulations did not yield any abundance estimates. Performance of 5 or 7 nights per route appeared to be similar to each other, so that using five nights per route would be a reasonable strategy. We then performed an additional 100 simulations only for five nights per route and the higher BFF density (0.015 BFF per ha), considering a total effort of 90, 120, 150, or 180 route-nights. These simulations indicated that bias and precision of abundance estimates improved with effort (Figure 1, Table 2).

FIELD PROTOCOL

General

Prior to field work, the above procedures should be used to determine the number and location of routes, and the number of nights of surveys to be performed per route. The goal of field surveys should be to complete the recommended number of surveys. If the actual total survey effort (nights*routes) differs from the planned number (possibly due to changes in volunteer numbers), the survey leader should adjust the number of nights per route while maintaining the target number of routes. Adjusting the number of nights per route may affect precision, while adjusting the number of routes entails a greater risk of bias. The field crew will be provided with a list of replacement routes in case a planned route is inaccessible or otherwise compromised. If a selected route contains potential BFF habitat, but it appears that it is unlikely to contain BFF (due to a lack of prairie dog sign), this route could be surveyed fewer times than other routes, but it must be surveyed for one night. The order in which routes are sampled may be adjusted to ease survey logistics. It may be convenient to survey all planned routes for one night before returning to those routes. If a route has been selected for surveys on a given night, it should be surveyed for an entire night. If it is not possible to complete a night of surveys (typically approximately eight hours), due to poor weather, vehicle breakdown, or other circumstances, data on start and end times should be recorded. If the route was surveyed for less than half a night, consider scheduling an extra survey. Model estimates will be more reliable if no animals are born, die, immigrate, or emigrate during the survey. Therefore, all surveys should be completed during a relatively short time period, such as a month. To facilitate comparisons across years, surveys should be conducted at approximately the same time each year. In addition, to increase precision of estimates, surveys should be conducted at a time of year when the number of captures is anticipated to be the highest and, if an estimate of recruitment desired, when dispersing young are available for capture.

Specific

A small number of observers (2 or 3) should be assigned to each route. Observers should be supplied with spotlights, a map indicating the start and finish point of their route, a GPS loaded with waypoints for the start and finish point of their route, trapping equipment, and a communication device (cell phone or radio). Observers should also be supplied with and carry spare spotlight bulbs, batteries, flashlights, pencils, fuses and tools to make repairs in the field. Inexperienced participants should be paired with experienced surveyors. At an appropriate time, observers should proceed to their assigned route. For road-side routes, observers should drive the length of the route at approximately 5-10 mph. Observers should continuously scan the area with spotlights, slowly sweeping the light forward and back, aiming the spotlight beam so that the top half of the beam falls above the horizon and the bottom half below. After driving the length of the route, observers should reverse direction and repeat the survey.

For off-road routes surveyed on foot, observers should use a GPS to navigate on foot along their route. Observers should stop every 30 m and scan the landscape with spotlights, slowly sweeping back and forth for 5 or 10 seconds so that the area is covered twice. Each survey point could be programmed into a GPS prior to surveys. After walking the length of the route, observers should reverse direction and repeat the survey.

When an animal is detected, observers should identify the animal using binoculars and behavioral characteristics. Observers can use behavior, orbital (eye) size, interorbital distance, and distance to the ground to differentiate among species. Among commonly encountered animals, BFF, badgers, young coyotes, and nighthawks have brilliant emerald green eyeshine. BFF eyeshine is typically close to the ground, remaining in one place, or bobbing up and down as the BFF looks out of a prairie dog burrow. When BFF do move, their eyeshine tends to bounce because of their bounding gait (Biggins et al. 2006). In addition, badgers have larger eyes and a greater interorbital distance, and the larger body of a badger often can be seen. Immature badgers may be mistaken for BFF. Coyote eyeshine may disappear as the animal trots away, and then reappear many meters away. Nighthawks will not run or enter a burrow, but may fly away.

If an animal other than a BFF is spotted, the observers should take a GPS waypoint (from the road if in a vehicle or from the walking route) and record the time, as well as a description of the behavior. If a BFF is detected, the observer should move directly towards the BFF and keep the spotlight focused on the animal. When working in teams of two, it is helpful to have one person hold a spotlight on the area/burrow where the BFF was sighted, while the second person gets gear and walks to the burrow. The spotlight should be directed slightly away from the animal, rather than directly into the eyes of the animal. If the eyeshine disappears from view, observers should scan to see if the animal has moved. If the BFF submerges into a burrow before its burrow can be identified, observers should stop and wait for the animal to re-emerge. If the BFF does not re-emerge, a flashlight can be used to inspect the closest burrows. Often the BFF will be in one of the burrows and can be spotted.

Once a BFF is located in a burrow, observers should use high visibility reflector posts to mark BFF-occupied burrows, readers and traps. Red works best and is highly visible from long distances. A strip of red reflective tape should be wrapped around the post so it can be seen from all directions, and the post should be pushed firmly at least six inches into the ground. Wind or

animals can knock over a reflector if it is not secure. Reflector posts should be placed a few feet from the burrow and not directly into the burrow mound. The observers should proceed to set traps according to the Black-footed Ferret Trapping Protocol.

Data Collection

Captured BFF should be transported to the processing station for marking, vaccination, and data collection. Specific instructions on handling captured BFF can be found in the Black-footed Ferret Immobilization Protocol. Additional data should be collected during surveys, regardless of BFF sightings. In particular, recording information on conditions that may affect the detection of BFF will improve estimates from SECR models. Relevant data would summarize the observers, the BFF, and environmental conditions. Observer data would include experience (e.g., two or more categories indicating the level of experience), number of observers, survey method (vehicle or foot), and number of passes (i.e., how many times the transect was completed within a night). Note that recent Department surveys have not recorded the number of passes. BFF data should include identification (if previously captured), age (adult or kit), sex, and location of capture (i.e., UTM coordinates). Data on environmental conditions would include moon phase, and possibly precipitation, temperature, wind speed, and similar. BFF seen, but not captured should also be recorded.

Reduced-Effort Protocol

SECR

If resources are not available for an intensive SECR survey during a given year, survey effort could be reduced. Fewer routes could be surveyed by selecting a smaller portion of routes generated by the GRTS process. Alternatively, a similar number of routes could be selected, with fewer survey nights per route. Decisions about the number of routes and the number of surveys per route should be made prior to the first survey, and not during the survey season. Reducing effort in this manner will reduce the precision of abundance estimates. However, if reduced-effort surveys are alternated with high-effort surveys, SECR models can "borrow" information among years, ameliorating the loss of precision.

Index

If resources are insufficient to capture BFF, or if it is judged that capturing BFFs is not in the animals' best interest, an alternative would be to count BFFs without capturing them. A count would not estimate abundance because of imperfect detection of BFF. Instead, the count would serve as an index to abundance. Note that there is no sound theory to estimate uncertainty of counts, and no significance tests available. If it is possible to assume that detection probability is constant across years (an untested hypothesis), then changes in the index would correspond to changes in abundance. In this case, the index could signal precipitous changes in abundance that might trigger a management response.

The major assumption of index surveys is that detection is constant across years. To achieve this constancy, surveyors should strive for consistency in survey methods, although not all survey features can be controlled (e.g., weather). As with SECR surveys, the number of routes and repeat surveys should be selected prior to surveys, with the specific routes selected from the

GRTS-generated list. If resources are constrained, only road-side routes could be selected. In either case, survey effort and location should be recorded.

Spotlighting of BFF should proceed as under SECR surveys. However, no BFFs need to be captured to calculate an index. Data collection should be similar to SECR surveys, although some information, such as sex, will not be available. If observers have reason to believe that an individual BFF was observed multiple times in one night, perhaps because observations were in close proximity, this should be noted. If BFF are observed on different nights, there is no need to determine if it is a unique individual or a repeat observation. Note that this survey method does not allow for vaccination of animals.

No Surveys

If resources are limited during a given year, and if program rules allow, a potential strategy would be to not perform any surveys, and to carry cost savings over to the next survey period. If managers judge that occasional, precise estimates of abundance are more useful than frequent, but imprecise measures, this strategy could be useful.

PROTOCOL IMPROVEMENTS

Increasing rates of capture and recapture will improve model estimates and help the project meet objectives. If animals have distinctive marks, it is possible to "recapture" them visually, without trapping them. Because approximately half of spotlighted BFFs are not captured (Biggins et al. 2006, Grenier et al. 2009), adding marks to BFF would increase recapture rates and improve estimates. BFF have been marked with dye (Grenier et al. 2009) and ear tags (Morley 2002). In particular, if ear tags are reflective, it may be possible to individually identify BFF from a distance (e.g. if unique color combinations are used), increasing recapture rates. If a BFF is identified visually, it may still be captured if necessary for some other program goal, such as vaccinations. Field trials testing the feasibility of these marks could lead to improved recapture rates and model estimates. However, it is also important that such marks are not lost during a survey and do not reduce survival of BFF.

DATA ANALYSIS

Software

A SECR analysis could be accomplished in various software programs, such as DENSITY, the Program R package 'secr', or WinBUGS. Software is listed in increasing order of modeling flexibility and technical skill required. DENSITY provides a graphical interface, but lacks useful capabilities, such as polygon detectors, density modeling, model averaging, hybrid mixture models, and data simulation, and survival estimation. Program R allows one to access 'secr' functions using the R language. This requires modest programming skills, but increases the flexibility and power of models. R includes additional capabilities, such as polygon detectors, density modeling, model averaging, and hybrid mixture models, but not survival estimation. WinBUGS is a modeling language that allows Bayesian analysis using Monte Carlo Markov Chain methods. It does not include any SECR functions. Instead, it provides a modeling language that a user can use to create likelihood statements describing SECR models. As such, it requires relatively advanced statistical and programming skills. Because users create their own

functions, it provides maximum flexibility and code could be created to estimate survival. In future years, it may be possible to automate at least some of the analyses.

Data

Data required for analysis includes the dimensions of the surveyed routes (described by the x-y coordinates of route vertices), and capture histories for animals that include animal ID, date and location of captures, relevant animal characteristics (age, sex, etc.), and relevant survey characteristics (moon phase, observer experience, etc.). It would also be useful to have a shapefile including relevant landscape features, such as paved roads or waterways, and the extent of potential habitat. The exact data format depends on the software used. Note that if using DENSITY, route polygons must be converted to a grid of virtual traps. The other software can analyze polygons. However, the only way to incorporate data on the distance between detected BFF and survey transects is to "discretize" the polygons (convert them to a grid of virtual traps), so the same data transformation may be used in Program R or WinBUGS.

Abundance

Assuming that Program R is used, the data would be used to create a trap object, a capture history object, and a mask object. The 'secr.fit' function would be used to specify different models of detection and abundance. For example, detection might be affected by moon phase, and density might be affected by paved roads. Various plausible models could be fit to the data, and model selection procedures used to select the best supported model. The 'region.N' function would be used to extract an abundance estimate from the secr object.

Survival

Estimating survival requires multiple years of data. Currently, only WinBUGS can be used to estimate survival as commonly understood (the probability that an individual that is alive at time t is also alive at time t+1). In contrast, DENSITY and Program R (DENSITY 5.0, secr 2.10) can only estimate abundance across multiple 'sessions' (i.e., years), not survival. Changes in abundance across sessions can be independent, or part of a trend. Trend estimated this way differs from a trend calculated from count data in that SECR analysis accounts for imperfect detection and can estimate the uncertainty around estimates. Estimating survival in WinBUGS would require developing functions that do not currently exist.

Recruitment

Recruitment can be measured by the number of offspring attaining a certain age. BFF young begin to disperse from their mothers in September and October (USFWS 2013). This dispersal period is both a milestone in the development of young and a period when detection of young improves. Therefore, surveys have typically been conducted in September and October, and recruitment can be measured as the number of young alive at that time. Estimates of recruitment (number of young alive) can be obtained from 'secr' functions in Program R. Currently (secr 2.10), the 'region.N' function does not provide estimates of abundance by group, but juveniles could be coded as a separate session, and abundance estimated for that session. Alternatively, the mixture model option in 'secr.fit' allows one to estimate the proportion of the population that is young. Note that estimates for a subset of the population will be less precise than for the entire population.

Index of Abundance

If no BFF are captured, data would consist solely of counts of BFF. The index to abundance would be based on the number of BFF seen per route per night. For example, if four routes were surveyed for three nights each and six BFF were seen, the index would be 0.5 BFF per route per night. If routes are surveyed for a portion of a night, this should be reflected in the tally of nights. To calculate changes in the index of abundance, the index should be compared to the previous index, for the same routes. For example, if the index on those four routes was 0.6 in the following year, then the index would suggest a 0.6/0.5 - 1 = 20% increase in abundance. Note that this procedure assumes that detection does not change between years, and it does not provide any estimate of uncertainty regarding changes in the index of abundance.

FUTURE REVIEW OF MONITORING PROTOCOLS

Given that the SECR analysis is a new approach to BFF monitoring, and the population may change through time, results should be reviewed regularly and the protocol should be adjusted as necessary. We recommend that the protocol is reviewed after each of the first three years of implementation, and then at least every five years thereafter. The current recommended protocol for estimating abundance is based on rough estimates of the density of BFF, detection of BFF, home range sizes, and the relative labor cost of off-road routes. If data indicates that these estimates were inaccurate, it may be appropriate to revise either total survey effort, or the number of survey nights per route. The protocol is intended to assist the Department in achieving management goals, such as assessing progress towards downlisting goals, and informing translocation decisions. If survey results are not adequate to inform current management decisions, or if new management scenarios arise, the protocol may be reassessed.

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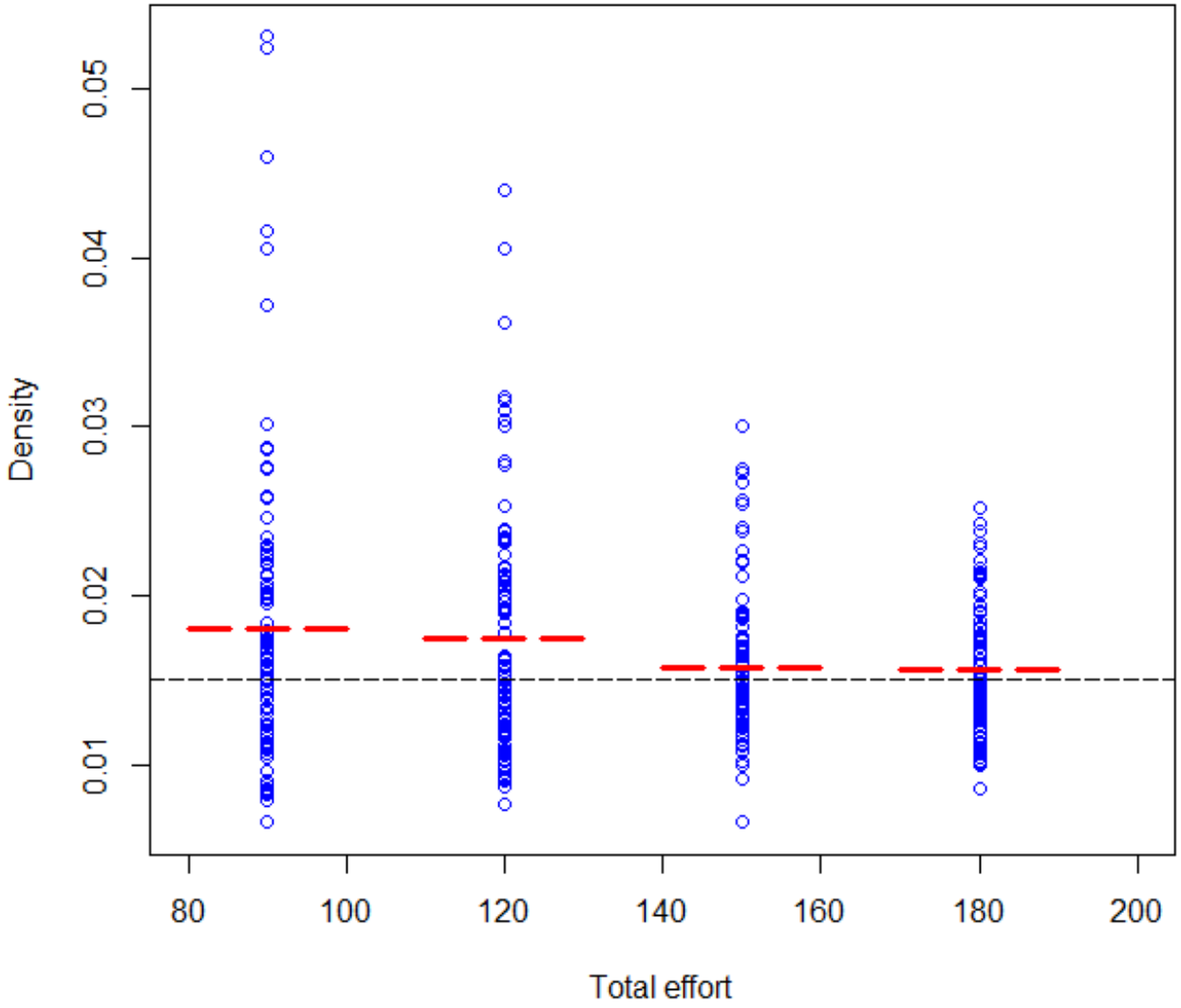
Table 1. Results of simulated ferret surveys. Scenarios differ in the number of routes surveyed, the number of survey nights per route, total effort (routes*nights), and the density of BFF. Each star represents the number, out of 20 simulations, in which no abundance estimate was generated. Root mean square error (RMSE) is a measure that declines as estimates become precise. RMSE can only be compared within a given effort and density.

Scenario	Sites	Nights	Effort	Density	RMSE	Low CI	Hi CI
1**	30	3	90	0.005	0.00369	0.0019	0.0291
2**	18	5	90	0.005	0.00455	0.0014	0.0354
3**	13	7	91	0.005	0.00301	0.0016	0.0238
4	40	3	120	0.005	0.00775	0.0022	0.0400
5*	24	5	120	0.005	0.00504	0.0023	0.0291
6	18	7	119	0.005	0.00299	0.0017	0.0250
7**	50	3	150	0.005	0.00652	0.0022	0.0403
8	30	5	150	0.005	0.00563	0.0029	0.0325
9	21	7	147	0.005	0.00308	0.0026	0.0162
10	30	3	90	0.010	0.01321	0.0054	0.0709
11	18	5	90	0.010	0.00307	0.0039	0.0263
12	13	7	91	0.010	0.00738	0.0049	0.0465
13	40	3	120	0.010	0.00655	0.0059	0.0313
14	24	5	120	0.010	0.00541	0.0060	0.0280
15	18	7	119	0.010	0.00470	0.0051	0.0258
16	50	3	150	0.010	0.00852	0.0061	0.0351
17	30	5	150	0.010	0.00296	0.0063	0.0217
18	21	7	147	0.010	0.00552	0.0066	0.0273
19	30	3	90	0.015	0.01069	0.0094	0.0542
20	18	5	90	0.015	0.00638	0.0068	0.0330
21	13	7	91	0.015	0.00822	0.0085	0.0426
22*	40	3	120	0.015	0.00749	0.0090	0.0317
23	24	5	120	0.015	0.00603	0.0090	0.0230
24	18	7	119	0.015	0.00624	0.0087	0.0342
25	50	3	150	0.015	0.00611	0.0093	0.0334
26	30	5	150	0.015	0.00560	0.0116	0.0307
27	21	7	147	0.015	0.00503	0.0099	0.0263

Table 2. Results of simulated ferret surveys. Each scenario simulated 100 times. In each scenario, density is 0.015 ferrets/ha, and each route is surveyed five nights. Scenarios differ in total effort (routes*nights). Density is mean estimate from simulations and abundance is based on 10,000 ha (24,700 acres) of BFF habitat containing 150 BFF.

Scenario	Effort	Density	Low CI	Hi CI	Abundance	Low CI	Hi CI
20	90	0.0181	0.0078	0.0458	181	78	458
23	120	0.0175	0.0092	0.0340	175	92	340
26	150	0.0158	0.0092	0.0273	158	92	273
28	180	0.0156	0.0099	0.0248	156	99	248

Figure 1: Results of simulated ferret surveys. Each scenario simulated 100 times. In each scenario, density is 0.015 ferrets/ha, and each route is surveyed five nights. Scenarios differ in total effort (routes*nights). Black line indicates true simulated density. Blue circles represent estimates from individual simulations and red lines represent means of the simulations.



Supplementary Code

Code written for Program R. First set parameters, then run simulations.

```
# Set parameters
num.rd <- 50            # number of road side units available
num.off <- 500          # number of off-road units available
size.rd <- 4            # size (area) ratio of road:off-road units
                        # off-road set to one, so 4 -> 4:1 ratio
cost.off <- 0.5         # labor cost ratio of road:off-road units
                        # off-road set to one, so 0.5 -> 0.5:1 ratio
num.samples <- 30       # desired number of sample units (routes)

# Simulations
out <- replicate(10000, {
xx <- rep(c(1,0), c(num.rd, num.off))
pp <- rep(c(size.rd,1/sqrt(1/cost.off)), c(num.rd, num.off))
yy <- sample(xx, num.samples, prob=pp)
mean(yy)
})
cat("Simulations recommend", round(mean(out)*num.samples), "road-side units
and", num.samples-round(mean(out)*num.samples),"off-road units.",'\n')
```

APPENDIX H: BLACK-FOOTED FERRET CAPTURE AND HANDLING PROTOCOL

PURPOSE

This protocol provides guidance for black-footed ferret (*Mustela nigripes*; hereafter BFF) capture, handling, marking, immobilization, and vaccination. Adherence to this protocol ensures the safety of the BFF and personnel. This protocol is based on material in Biggins et al. (2006) and BFFRIT (in prep).

BACKGROUND

Once located through spotlighting, the capture of individuals allows project biologists to mark and vaccinate individuals against disease. Mark and recapture is the primary technique used to determine survival rates for BFF. Although long-term survival rates are relatively low, capture events in the spring are conducted to assess winter survival of BFF, while fall events capture dispersing juveniles and determine population size going into winter. The most effective and reliable method of marking BFF in the wild is by passive integrated transponder (PIT) implants, also referred to as a transponder chip or PIT tag.

PROTOCOL

EQUIPMENT

1. Traps
    a. Tomahawk live trap– 36L x4W x 4H/1x1 12 Gauge Model 4436.
    b. A six-inch tunnel insert attachment.
    c. A canvas, burlap, or wool cloth wrap.
2. Anesthesia
    a. Machine
    b. Isoflurane
    c. Masks
    d. Induction chamber
    e. Oxygen
    f. Charcoal anesthetic scavenging system
2. Processing
    a. Syringes and needles
    b. Flea comb and/or tweezers
    c. Hemastat
    d. Thermometer
    e. Stethoscope
    f. Fecal loop
    g. Swabs
    h. Tubes, cryovials, nobuto strips

i. Whirl packs
j. Scale
k. PIT tags and injector and scanner
l. Permanent Marker
m. Lubricating eye ointment

3. Supportive care and medications
    a. Heating pad and hot water bottles
    b. Replacement fluids: saline, lactated ringers, normosol, or plasmalyte
    c. Distemper vaccine
    d. Plague vaccine
    e. Penicillin or ceftiofur
4. PPE and disinfectant supplies
    a. Gloves
    b. Masks
    c. Chlorexidine solution

FIELD PROTOCOL

1. Trapping.
    a. Timing
        i. Do not trap when temperatures and/or wind chill is below 0° F.
    b. Inspect all traps and ensure they are working properly.
    c. Wrap the trap and tunnel insert with burlap, canvas, or wool so no light get through.
    d. Set the trap.
        i. Insert hand, palm up, into the round portion of the trap and push the trap door to the top. Using index finger, find the trigger and pull until it is just under the door. Push the trigger back to the edge of the door for a light-triggered set.
        ii. Firmly push the rounded tunnel-insert end into the burrow.
        iii. Once in the burrow, move trap back and forth to settle the tunnel-insert into the dirt. The trap should feel firmly set so there will be no trap movement.
        iv. Ensure there is a clear path from the burrow into the trap, with the treadle on the bottom.
        v. Confirm the rear door is locked and secured with a clip.
    e. Increase trapping success options.
        i. Minimize time spent at burrow - Set one trap, place reflector, record waypoint with GPS unit, and leave area.
        ii. Set one trap and plug nearby burrows.
            1. Always record number of plugs set.
    f. Check traps at least hourly.
        i. Inspect full length of trap.
        ii. Record time on the data sheet.
2. Handling
    a. Personnel

i. Must be certified by the USFWS.
ii. Must be free of influenza-like symptoms.
iii. Must use personnel protective equipment.
iv. A minimum of two personnel are required.
    1. Data Recorder.
        a. Record routine processing data, including any drug or vaccinations, transponder number implanted, and other pertinent data.
        b. Monitors respiration.
        c. Assist Processing Lead.
        d. Photo document as needed.
    2. Processing Lead.
        a. Processes animal.

b. Before anesthesia.
    i. Examine for signs of injury or extreme stress.
    ii. Scan for PIT Tag.
        1. If PIT tagged, administer booster shots of plague if two weeks or later from the first vaccination.
        2. Prepare the animal for release.
    iii. Determine sex.
        1. Female.
            a. Typically smaller than male.
            b. Thin, faint brown/black continuous medial line on ventral side, extends caudally from mid-belly to vulva.
        2. Male.
            a. Typically larger than female.
            b. Dark brown/black ventral line from mid-belly, extending caudally and widening near penis.
            c. Testicles may be apparent.

c. Sedation with isoflurane.
    i. Use induction chamber and handling cage.
    ii. Oxygen flow rate 2.5-3 liters/minute.
    iii. Isoflurane at 4%.
    iv. Do not move chamber to check response. Induction time is two minutes.
    v. Once fully sedated, remove from induction chamber and lay flat on its back on processing table.
    vi. Place mask fully over face ensuring a tight seal.
    vii. If animal is limp, turn the isoflurane down to 3% and oxygen down to 2 liters/minute.
    viii. For oral or prolonged procedures a 2.5-3.5 mm cuffed endotracheal tube is required.
    ix. Monitored pulse oximetry by tongue, ear, tail clamp, or rectal probe.

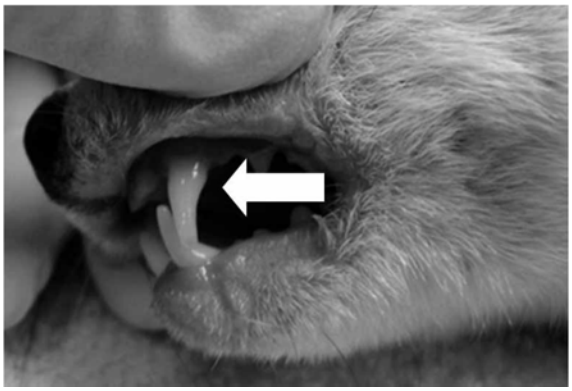
d. Processing.
    i. Apply saline/artificial tear ophthalmic lubricating ointment to eyes.
    ii. Monitor respiration and heart rate.
        1. Adult heart rate.

a. 220-250 bpm (awake).
b. 180-225 bpm (under Isoflurane).
c. 75-150 bmp (under medetomidine/ketamine).

2. Respiratory rate – 20-70 breaths/min.

iii. Check body temperature every 10 min while processing, and post-processing temperature.
1. Body temperature – 99.0 – 102.0 F.

iv. Sex/Age Determination.
1. Gum recession, wear on teeth, replacement of deciduous teeth with permanent teeth per Santymire et al. (2012) (Figures 1 and 2).
a. Adult dental formula: incisors 3/3, canines 1/1, premolars 3/3, molars ½ for a total of 34.

Figures 1 and 2. Juvenile on left, note no gum recession; adult on right, base of canine parallel indicating gum recession. (Santymire et al. 2012).

2. A female with apparent and lactating nipples is an adult.
3. Male body size relative to the time of year distinguishes between a juvenile and adult.
a. BFFs are adult-sized by 95-100 days of age.

v. Marking
1. Mark an X on BFF neck with a permanent marker to quickly identify the animal in a recapture.
2. PIT tags should be injected/placed with a needle that has been sterilized between animals or purchased in preloaded applicators.
3. Insert PIT tag between the shoulder blades.
a. Scan to verify the number.

vi. Administer vaccines.
1. BFFs are uniquely sensitive to vaccines and should NOT be vaccinated with any modified live product.
2. BFFs can be vaccinated in trap without anesthesia by grasping a fold of skin through the trap.
3. Prepare vaccine syringe.
4. Administer vaccine subcutaneously by inserting the needle under the skin and injecting the proper amount of vaccine, taking care not to poke the needle through the tented skin.
a. Canine Distemper Virus (CDV).
i. Merial's recombinant Pure Vax Ferret Distemper vaccine.

1. 1 cc for vaccination.
2. No boosters given.
3. Vaccine should be kept frozen until used.
4. Not currently available.

ii. Alternative Merial Recombitek CDV (approved for dogs).

b. Plague (F1V).
   i. Only administered at USFWS National Black-footed Ferret Conservation Center (NBFFCC) or field sites
   ii. Produced specifically for BFFs at the USFWS NBFFCC. All doses must be ordered directly and shipped refrigerated for overnight delivery.
   iii. Vaccine has a very limited shelf life of only two weeks.
   iv. Two injections, 3-6 weeks apart.
      1. First dose – 0.5 ml subcutaneous.
      2. Second dose – 0.25 ml subcutaneous.
         a. Repeat first dose if second capture more than three months after 1st dose.

c. Rabies
   i. Single dose Imrab 3 by Merial given subcutaneous.
   ii. No boosters given

vii. Sample collection.
   1. Disease/Parasite sampling.
      a. Swab throat and place swab in glycerol/media.
      b. Collect fecal sample.
         i. Fecal loop.
         ii. Swab thermometer or rectum.
         iii. Small amounts or a swab are placed in glycerol/media.
         iv. Large amounts are placed in a whirl pack.
      c. Swabs, tubes, and envelopes should be labeled with the animal's id number.
   2. DNA sampling.
      a. Pluck 10 to 20 hairs from back near base of tail and place in envelope.
      b. Hair envelopes should be labeled with the animal's id number.
   3. Blood collection options
      a. Clip a back toe nail and collect two nobuto strips.
      b. Collect 2 to 3 cc from the jugular/cranial vena cava.
         i. Alternative sites for small quantities for iSTAT blood analyzer.
            1. Cephalic vein.

2. Caudal vein.
3. Femoral vein.

c. Blood should be placed in a red top or tiger top tube and a purple/lavender topped tube labeled with the animal's id number.
d. Tubes should be allowed to clot for 30 min then spun at 3500 rpm for 10 min.
e. Serum should then be removed and placed in cryovial.
f. Serum, swabs, hair, and nobutos should be sent to the Wildlife Health Program for archiving or submission for diagnostic testing:

Anne Justice-Allen, Wildlife Health Program Supervisor
Arizona Game and Fish Department
5000 West Carefree Highway
Phoenix, AZ 85086
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e. Administer antibiotics .
   i. Injectable procaine penicillin g 300,000 units/cc, 0.4cc subcutaneous.
   ii. Or Ceftiofur 50mg/cc, 0.1cc subcutaneous.
f. Weigh BFF.
   i. Adult Male: 850-1000 gm.
   ii. Adult Female: 650-850 gm.
g. Post-processing.
   i. Place BFF in a carrier.
   ii. Continue to monitor until BFF awakens.

3. Release.
   a. Release at the capture burrow or an adjacent burrow.
   b. Leave a small amount of prairie dog with the released animal.
   c. Point the square end of the trap at the burrow without people or vehicles in the direct sight of the BFF and open the rear door.
   d. It is not uncommon for the BFF to enter the burrow slowly after anesthesia.
      i. Crumple a plastic or paper bag to coax the BFF out of the trap.
      ii. Open the carrier door and grab the towel or pad from inside the carrier and pull it out.
      iii. Tilt the carrier towards the burrow and the BFF will slide.

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APPENDIX I: BLACK-FOOTED FERRET RELEASE PROTOCOL

PURPOSE

This protocol describes procedures related to the release of black-footed ferrets (*Mustela nigripes*; hereafter BFF) either from captive-bred progeny or through translocation efforts.

BACKGROUND

Releases and translocations are an essential component to the re-establishment, management, and recovery of BFF. This *Management Plan for the Black-footed Ferret in Arizona* describes survey efforts, population levels, management actions, and environmental factors that must be achieved before the Arizona Game and Fish Department will release or translocate BFF. This protocol is merely a summary of the necessary data needed (**Bolded Text**) and the requirements to release BFF as described within this plan.

PROTOCOL

1. Source population.
    a. Captive-bred
        i. Greater than 30 individuals at a 2:1 female to male ratio can be used to establish a new population in a Suitable MA.
        ii. Less than 30 individuals or less than a 2:1 female to male ratio can be used to augment populations in an Active MA, if:
            1. **BFF Density Estimates** in the Active MA does not exceed one female per 125 GPD-occupied acres as determined by the **GPD Correlated Detection Occupancy Models** or **GPD Perimeter Mapping**, or
            2. **BFF Monitoring Survey** determines a gap in occupancy.
    b. Translocation
        i. **BFF Monitoring Survey** shows a population increase over three years.
        ii. **BFF Monitoring Survey** shows the population exceeds the expected number of BFF family groups based upon the average GPD-occupied acres as determined by the **GPD Correlated Detection Occupancy Models** or **GPD Perimeter Mapping**.
            1. **BFF Density Estimates** exceeds one female per 125 GPD-occupied acres as determined by the **GPD Correlated Detection Occupancy Models** or **GPD Perimeter Mapping,** and greater than 30 individuals at a 2:1 female to male ratio can be harvested to establish a new population in a Suitable MA.
                a. Animals will only be harvested in the fall.
            2. **BFF Density Estimates** exceeds one female per 125 GPD-occupied acres as determined by the **GPD Correlated Detection**

Occupancy Models or **GPD Perimeter Mapping,** and less than 30 individuals or less than a 2:1 female to male ratio can be harvested to augment populations in an Active MA.

a. Animals will only be harvested in the fall.

iii. Latest **BFF Monitoring Survey** does not show a decline from the previous year.

2. Release area.
   a. Establishment of a new population.
      i. **GPD Correlated Detection Occupancy Models** or **GPD Perimeter Mapping** shows a minimum 5,540 GPD-occupied acres.
         1. GPD Population trends are stable or increasing.
         2. **GPD Density Mapping** conducted to inform release areas.
         3. **Predator Population Trend Surveys** conducted one-month prior to any release.
            a. **Predator Management** informed by Predator Population Trend Surveys.
         4. **Burrow Dusting** occurs two-weeks prior to release.
      ii. **GPD Perimeter Mapping** shows a minimum 5,540 GPD-occupied acres within a complex.
         1. GPD Population trends are stable or increasing.
         2. **GPD Density Mapping** conducted to inform release areas.
         3. **Predator Population Trend Surveys** conducted one-month prior to any release.
            a. **Predator Management** informed by Predator Population Trend Surveys.
         4. **Burrow Dusting** occurs two-weeks prior to release.
   b. Augment existing population.
      i. **BFF Monitoring Survey** shows a gap in distribution.
      ii. **BFF Density Estimates** does not exceed one female per 125 GPD-occupied acres.
      iii. **GPD Correlated Detection Occupancy Models** or **GPD Perimeter Mapping** shows population trends are stable or increasing.
      iv. **GPD Density Mapping** conducted to inform release areas.
      v. **Predator Population Trend Surveys** occurs one-month prior to any release.
         1. **Predator Management** informed by Predator Population Trend Surveys.
      vi. **Burrow Dusting** occurs two-weeks prior to release.
3. Post-release monitoring.
   a. **BFF Monitoring Survey** 30 days post-release.
   b. **GPD Density Mapping** conducted annually for first five years following the last release.
      i. Reduced to every three years after initial five year period.
   c. Annual **BFF Monitoring Survey** three times annually (mid-spring, mid-summer, mid-fall) for first five years following the last release.
      i. Reduced to every three years after initial five year period.

APPENDIX J: BLACK-FOOTED FERRET BREEDING PROTOCOL

PURPOSE

This protocol outlines the procedure for breeding black-footed ferrets (*Mustela nigripes*; hereafter BFF), including assessment of reproductive status, pairing, and follow-up.

BACKGROUND

Arizona was the first reintroduction area to begin an on-site breeding program in pre-conditioning pens. From 1997-2000, BFF were bred in the pre-conditioning pens. Females and kits were released when the kits reached the typical dispersal age of approximately 120 days. However from 1996-2000, no wild born kits were found. In 2001, the release strategy was modified to include spring releases of mid-gestation females to coincide with prairie dog births, with the hopes that wild born kits would have a higher survivorship rate due to the ability to find easier prey (i.e. young prairie dogs) than captive-raised kits released in the fall. The strategy proved successful as seven wild born kits were discovered in 2001. Releasing mid-gestational females also reduced resource and personnel costs (i.e. personnel for husbandry and feeding, food costs, etc.), making the strategy more economical.

PROTOCOL

EQUIPMENT

1. Pipette, slides.
2. Saline solution.
3. Cytological fixative, stains (if on-site staff will read slides).
4. Microscope.

FIELD PROTOCOL

1. Assess individual reproductive status.
    a. Males.
        i. Weekly monitoring for firmness of the testicles.
        ii. Males can be paired with a female when testicles are firm.
            1. Testicles will remain firm for approximately 30 days.
            2. If a male is to be paired more than once, a minimum of three days between pairings is required.
    b. Females.
        i. Weekly monitoring for a size change of the vulva.
        ii. Estrus is reached when there is a change in vulval measurement >14mm.
            1. Vaginal lavage is obtained using sterile plastic 1-ml syringe and plastic pipette tip.

a. Gently insert pipette approximately 1.0-1.5 cm into vagina until slight resistance.
b. Flush 0.05-0.1 ml sterile physiologic saline, and aspirate several times.
c. Expel contents onto a clean glass slide
d. Spray with cytological fixative and allow to air dry
    i. Stain
    ii. Females can be paired when 90% of cells are cornified epithelial.

2. Pairing.
    a. Pairing occurs within days after both individuals are ready.
    b. Introduce male to female's nest box.
    c. Leave paired for three days
    d. Remove male.
    e. Examine the back of female's neck for an orange stain to confirm breeding.
    f. Perform a second lavage on female 8-9 days after separation from male.
        i. Breeding is successful if second lavage shows a decrease in epithelial cells.
3. Release of mid-gestational females
    a. Females should be released within two weeks of confirmed breeding.
    b. Males and females can be released in proximity if breeding status is uncertain.

LITERATURE CITED

Williams, E.S., E.T. Thorne, D.R. Kwiatkowski, K. Lutz, and S.L. Anderson. 1992. Comparative vaginal cytology of the estrous cycle of black-footed ferrets (*Mustela nigripes*), Siberian polecats (*M. eversmanni*), and domestic ferrets (*M. putorius furo*). Journal of Veterinary Diagnostic Investigation 4:38-44.

APPENDIX K: PREDATOR POPULATION TREND PROTOCOL

PURPOSE

This protocol provides guidance for assessing predator population trends in black-footed ferret (*Mustela nigripes*; hereafter BFF) populations.

BACKGROUND

The primary predators of BFFs are coyotes (*Canis latrans*), badgers (*Taxidea taxus*), great horned owls (*Bubo virginianus*), kit fox (*Vulpes macrotis*), and gray fox (*Urocyon cinereoargenteus*; Biggins et al. 2006). These species occupy current and potential BFF habitat, but population abundance of each species is unknown due to their low density and large home ranges, therefore the predator's effect on the BFF population is unknown. This protocol describes methods for generating trends in predator abundance. Trends can be determined if the field protocol is constant. The resulting information, along with information on the changes in BFF abundance, can inform predator management decisions.

In constructing a trend survey, it is important to define the appropriate metric. In the case of predators, the metric could be either defined by the number of individual predators observed, or the total number of predators observed. In the first, an individual is only be counted once, while the second would allow an individual to be counted multiple times. Due to difficulties in identifying individual unmarked animals, this protocol uses total observations.

This protocol recommends performing simultaneous spotlight surveys for predators in conjunction with BFF surveys. Predators are commonly detected during spotlight surveys, and a simultaneous survey will capture the appropriate data to determine predator population trends.

PROTOCOL

FIELD PROTOCOL

1. Conduct BFF Population Monitoring Protocol.
2. Once a predator is identified.
    a. Record time.
    b. Record location NAD 83 UTMs from a GPS.

ANALYSIS

A simple index to abundance can be defined as the number of observations of predators per route per night. For example, if four routes were surveyed for three nights each (12 total surveys) and coyotes were observed six times, the index would be 0.5 coyote observations per route per night. If routes are surveyed for a portion of a night, this should be reflected in the tally of nights. For

example, if a typical survey lasts eight hours, then a 4-hour survey should be recorded as ½ of a night. Given that the threat to BFFs and appropriate management methods vary among predators, a separate index should be calculated for each predator. To calculate changes in the index of abundance, the index should be compared to the previous index. For example, if the index was 0.6 in the following year, then the index of abundance has increased by 0.6/0.5 - 1 = 20%. Changes in the index should be calculated using the same set of routes in each year. In particular, detection and behavior of predators could differ in unknown ways between vehicular and pedestrian surveys. If detection does differ among routes, then changes in the routes used to calculate the index could induce changes in the index, even if predator abundance is constant.

Note that this protocol assumes that detection does not change between years, and it does not provide any estimate of uncertainty regarding changes in the index of abundance. Under the assumption that detection probability differs among predators, the level of the index cannot be compared across predator species. If managers desire a more robust index of population trend, an expanded protocol and increased field effort would be needed to estimate changes in detection (e.g., due to changes in vegetation cover).