

**Study Plan**

**Potential Causes of Pronghorn Decline in Arizona**

**June 13, 2002**

**Prepared by:**

**Shelli A. Dubay**

**Arizona Game and Fish Department**

**Research Branch / WMRS**

**2221 W. Greenway Rd.**

**Phoenix, AZ 85023**

**Federal Aid Project Number W-78-R**

## **Justification for Research**

Over the last 15 years, pronghorn have declined in Arizona. In 1987, the population of pronghorn was estimated at near 12,000 individuals, but the population declined to less than 8,000 by 2000 (Arizona Game and Fish Department 2001). Of particular concern is the Anderson Mesa pronghorn population; over 80 % of the pronghorn perished during a blizzard in the winter of 1967 (Neff and Woolsey 1979). Since that time, pronghorn numbers have fluctuated with aerial surveys showing between 153 animals in 1968 and 1,185 animals in 1985 (Neff and Woolsey 1979; AGFD unpublished data). In 2000, 220 pronghorn were surveyed on Anderson Mesa (AGFD unpublished data). Several potential causes of pronghorn decline have been identified, including diet overlap with cattle and sheep, fences that prevent movement, human development, water availability, predators, parasites and diseases, and nutritional concerns (Lee et al. 1998). Our goal is to determine potential causes of decline by investigating diseases, nutrition, water availability, presence of predators, and fawn hiding cover of pronghorn herds around Arizona. This project began in July 2001 and will continue through June 2005.

## **STUDY SITES**

A system of paired sites in 3 grassland regions of Arizona has been chosen based on mean pronghorn fawn recruitment in game management units (GMU) over the last 10 years. Pronghorn fawn recruitment data from 1991 through 2000 were obtained from the Game Branch, AGFD and mean recruitment over the 10-year period was calculated by GMU. In order to select sites with chronically poor recruitment, GMUs with a mean below approximately 15 fawns per 100 does (i.e., the low sites) were identified. GMUs with  $\geq 25$  fawns per 100 does on average or areas where mean fawn recruitment exceeded that in the low sites by at least 10 fawns per 100 does (i.e., the high sites) were also identified, and fawn recruitment was mapped by GMU (Fig. 1). A pronghorn habitat model was consulted to determine where pronghorn habitat exists in each GMU (Ockenfels et al. 1996). Once units and pronghorn habitat areas were identified, game specialists in each regional office were contacted and individual study sites within each unit were chosen based upon fawn recruitment and pronghorn use in individual GMUs.

Sites in GMUs 1 and 2B in Region I, GMUs 5B and 8 in Region II, and GMUs 30A and 36B in Region V were chosen.

### **Region I Sites**

GMU 1 is an ideal high recruitment site for several reasons. First, this unit had a mean fawn recruitment of 25.9 fawns per 100 does over the past decade, and it currently harbors a healthy pronghorn population over much of its area. In addition, the AGFD purchased property, the White Mountain Grassland Wildlife Area, in this unit to set aside habitat for wildlife, and therefore access on this site is not a limiting factor. Predator control practices have not been employed on the Wildlife Area since 1999. GMU 2B is geographically close, approximately 20 km away from the Unit 1 site, but had a mean fawn recruitment of 15.7 fawns per 100 does, suggesting that some factors other than location are playing roles in low reproductive success in this unit. Predator control has not occurred in the Coyote Hills area in GMU 2B since 1997, so this unit seems to be a logical site to oppose the Wildlife Area in GMU 1 in this project. Both GMUs have resident pronghorn herds, and grazing has occurred regularly on the sites in the last decade.

### **Region II Sites**

Pronghorn in GMU 8 have been studied by AGFD for several years, and radio-collared pronghorn currently inhabit the Garland Prairie portion of the GMU from March through September (Ockenfels et al. in preparation). In addition, a mean fawn recruitment of 37.1 fawns per 100 does has been calculated separately for the Garland Prairie site in GMU 8 (J. Goodwin, AGFD unpublished data). Sheep and cattle have been grazed on Garland Prairie during the last decade, and predator control has not been practiced on the site. GMU 5B encompasses Anderson Mesa, an area with very healthy and visible pronghorn populations from the 1960s through the 1980s (Neff and Woolsey 1979, AGFD unpublished data). During the last decade, however, a mean fawn recruitment of 11.7 fawns per 100 does has contributed to a significant decline in pronghorn populations on the Mesa. Overgrazing by cattle, juniper encroachment, high fawn predation rates, and limited movement corridors have all been proposed as reasons for the decline (Lee et al. 1998). Predator control has not occurred on Anderson Mesa

since 1989 (J. Goodwin, AGFD personal communication). Pronghorn populations on Anderson Mesa and Garland Prairie are migratory, and those on Garland Prairie move over 60 km seasonally (AGFD unpublished data).

### **Region V Sites**

GMU 34B has a mean of 23.2 fawns per 100 does over the last 10 years, with considerable variability in recruitment (2-51 fawns per 100 does). Grazing occurs throughout the Empire Ranch, and the Bureau of Land Management owns the land and BLM employees are eager to work with us on this project. Buenos Aires National Wildlife Refuge is located in GMU 36B and fawn recruitment averaged 12.9 fawns per 100 does over the last decade. Predator control and grazing have not occurred on site since 1985, when the refuge was founded. Prescribed burning has been employed to improve habitat for masked bobwhite quail (*Colinus virginianus ridgwayi*), but resultant forb growth also benefits pronghorn. Pronghorn were re-introduced into the Empire Ranch in 1984, and Buenos Aires National Wildlife Refuge received pronghorn in 1987. Both 34B and 36B currently harbor resident pronghorn populations with seemingly ample pronghorn habitat.

## **COMPONENT 1- DISEASE SURVEILLANCE**

### **Background**

Pronghorn have shown antibody titers against many bovine diseases, but herds have been influenced by few infectious agents and the effect that these infections have on pronghorn populations is largely unknown (Lance and Pojar 1984). The viral hemorrhagic diseases, bluetongue (BTV) and epizootic hemorrhagic disease (EHD), have been implicated in deaths of pronghorn in Wyoming, and over 3,200 pronghorn died during a BTV epizootic in eastern Wyoming in 1976 (Thorne et al. 1988). In 1984, 288 pronghorn carcasses were recovered and BTV was isolated from necropsied animals, but 600 to 1,000 pronghorn were estimated to have perished from bluetongue infection during the epizootic (Thorne et al. 1988). During summer 2001, EHD was identified as the cause of death in 1 pronghorn necropsied at the Wyoming State Veterinary Laboratory, but EHD is suspected as the cause of death in 3 additional pending cases (W.

Cook, Wyoming Game and Fish Department, personal communication), suggesting that EHD is currently active in the western United States. In addition, 2 mule deer from near Prescott, Arizona were diagnosed with hemorrhagic disease upon necropsy during September 2001. Bluetongue and/or EHD viruses are likely the cause of death for these deer.

Biting gnats of the genus *Culicoides* transmit both EHD and bluetongue viruses to ruminants, and epizootics often occur in late summer and early fall, with dead animals typically being found near water. *Culicoides* spp. require water for breeding and development, so epizootics of hemorrhagic disease often follow periods of hot, dry weather that cause gnats and ruminants to concentrate near watering holes (Nettles and Stallknecht 1992).

Clinical signs of hemorrhagic disease include listlessness, lack of appetite, sudden death when disturbed, and little reaction to humans (Thorne 1982*a,b*). Bluetongue is a fast-acting virus in pronghorn; experimental infection caused death in pronghorn approximately 8 days after inoculation (Thorne 1982*a*). Hoff and Trainer (1972) infected 4 pronghorn with the bluetongue virus subcutaneously. Two pronghorn possessed antibodies against bluetongue prior to experimental infection, while the other 2 did not. The 2 individuals that had prior antibodies did not develop clinical signs of disease, but the 2 pronghorn without antibodies prior to inoculation developed clinical signs and died 7 and 8 days later.

Throughout Arizona, pronghorn seem to be exposed to bluetongue virus periodically, but the effect of the disease on pronghorn populations has not been elucidated. Bluetongue antibody titers have been detected in pronghorn in Arizona (Heffelfinger et al. 1999), but the degree of disease resistance provided to exposed pronghorn herds is unknown. Heffelfinger et al. (1999) found that 79 % of 288 hunter-killed pronghorn sampled from numerous sites in Arizona had antibodies against bluetongue virus. Given that hemorrhagic disease epizootics occur in late summer and early fall, coinciding with the pronghorn breeding season, infection could cause behavioral or physiological changes, thereby decreasing breeding success and therefore fawn recruitment. Thorne et al. (1988) documented a fawn to doe ratio of 47:100 does 1

year after a bluetongue epizootic, while a ratio of 101:100 does was calculated outside the area of the epizootic.

The viral hemorrhagic diseases are the most likely agents to cause outright disease in pronghorn, but certain additional infectious organisms could influence thriftiness and reproduction in pronghorn herds. Pronghorn populations on Hart Mountain National Antelope Refuge in Oregon have shown steady decline in the 1990s, and a low fawn to doe ratio (1:100 does) has been identified as a contributing factor to the overall decline on the refuge (Dunbar et al. 1999). As part of an overall health assessment, 104 fawns, 40 adult does, and 9 adult males were evaluated for nutritional and disease status. Sera from adult females were tested for antibodies against several bovine diseases, including *Brucella* sp., *Leptospira* sp., bluetongue virus, EHD virus, bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PI3), infectious bovine rhinotracheitis virus (IBR), and bovine viral diarrhea virus (BVD). Antibodies against PI3 were found in 67 % of the animals tested, bluetongue antibodies were detected in 35% of the animals, while antibodies against EHD were found in 30 % of the animals. Bluetongue and EHD could dramatically influence pronghorn populations. To date, PI3 infection has not been implicated in an epizootic, but infection could increase susceptibility to other infectious agents or cause behavioral changes in affected individuals (Dunbar et al. 1999). Thorsen et al. (1976) isolated PI3 from nasal swabs from 3 of 50 pronghorn sampled in Alberta, and they suggested that PI3 causes infection in pronghorn. Clinical signs of PI3 infection in pronghorn have not been identified (Lance and Pojar 1984).

*Chlamydia psittaci* is an intracellular bacterium that causes abortion and vesiculitis in domestic sheep (McCafferty 1990). In 1992, the AGFD captured 37 pronghorn from 2 populations in Arizona to radio mark animals for a movement pathways project (O. Alcumbrac, veterinarian, personal communication). Cervical swabs and preputial washes from pronghorn were analyzed for bacterial infection and blood was taken to measure nutritional parameters, including copper and selenium in serum.

*Chlamydia* sp. was identified from cervical and preputial samples in approximately 80 % of the individuals sampled. In addition, serum copper levels were below the adequate range for domestic livestock. Interestingly, copper deficiency predisposes domestic sheep to *Chlamydia* sp. infection. Given that *Chlamydia* sp. cause reproductive problems

in domestic sheep (McCafferty 1990), copper deficiency and *Chlamydia* sp. infection could influence fawn recruitment in Arizona (O. Alcumbrac, personal communication).

### **Objectives**

1. Collect pronghorn blood samples from hunters in study areas each fall for 4 years.
2. Test blood samples for antibodies against various diseases that may cause epizootics in pronghorn.
3. Document areas where particular disease agents have been active over the course of the study.
4. Determine which disease agents could potentially influence pronghorn populations in Arizona.
5. Determine if antibody levels from pronghorn in GMUs with high fawn recruitment differ from levels in pronghorn from GMUs with low recruitment.

### **Approach**

Blood tubes will be mailed to hunters in GMUs 1, 2B, 5B, 8, and 30A to facilitate blood collection from pronghorn. A letter explaining blood collection protocol will be included in the mailing (Appendix A). AGFD employees will collect blood samples from hunters via check stations, and wildlife managers will collect samples during routine field activities. Blood will be centrifuged and serum will be separated and sent to the University of Arizona Veterinary Diagnostic Laboratory, Tucson, Arizona for appropriate antibody tests.

### **Predictions and Research Hypotheses**

1. Arizona pronghorn will show antibody titers against several bovine pathogens, particularly viruses that cause epizootic hemorrhagic disease and bluetongue.
2. Disease exposure will occur in all sites, but exposure levels will differ.
3. Pronghorn in GMUs with low fawn recruitment will have increased disease exposure when compared to pronghorn sampled from GMUs with high fawn recruitment.

### **Methods for Objective 1**

Individuals with pronghorn hunt permits in each study area will be identified after the fall hunt drawing is completed, and mailing labels for each GMU will be acquired from Customer Service at the Phoenix AGFD office. A blood tube (Falcon 50 ml sterile centrifuge tube) and accompanying blood collection protocol will be mailed to each hunter along with a list of drop-off locations (Appendix A). A map of the GMU will be placed on the back of the letter and the hunters will be asked to mark kill location on the map. Each sample will be labeled with hunt GMU, date, sex of animal, and a sample number, consisting of hunt unit and chronological number of sample from that unit (i.e., 5B-1, 5B-2, etc.). Check stations and drop-off locations will differ with site, but in general, check stations will run for the entire length of the hunt in each site. Research personnel and regional biologists will collect samples in the field.

### **Methods for Objective 2**

Blood samples will be centrifuged at 1,500 RPM for 12-15 minutes and serum will be separated from cells with 1 cc tuberculin syringes without needles. Sera will be labeled with information outlined above and refrigerated until either hand delivered or mailed to the University of Arizona Veterinary Diagnostic Laboratory (AVDL; 2831 N. Freeway, Tucson, AZ 85705). When mailing samples, an insulated mailing box from the diagnostic lab will be used, and samples will be mailed overnight via Federal Express.

Sera will be tested by AVDL for antibodies against known pronghorn pathogens, namely parainfluenza 3 (PI3), *Chlamydia psittaci*, epizootic hemorrhagic disease virus (EHD), and bluetongue virus (BTV). In addition, we will determine if pronghorn harbor antibodies against bovine viral diarrhea virus (BVD), infectious bovine rhinotracheitis virus (IBR), and bovine respiratory syncytial virus (BRSV) to determine if these pathogens are indeed circulating in Arizona pronghorn populations. *Chlamydia* sp. antibodies will be detected via complement fixation, and antibodies against IBR, BRSV, PI3, and BVD will be detected via serum neutralization. Antibodies against bluetongue and EHD will be detected via agar immunodiffusion. Because viruses that cause bluetongue and EHD are similar, serum neutralization tests for BTV and EHD will be performed on sera that are positive via agar immunodiffusion. Serum neutralization will allow us to determine if antibodies against EHD, BTV, or both are present in the sera.

### **Methods for Objectives 3 and 4**

In order to determine geographic distribution for disease exposure, hunters will be asked to mark kill location on a map and antibody levels will be mapped accordingly. Such mapping will allow us to elucidate trends in disease exposure by year and by site. In addition, antibody titers can be compared to specific Geographic Information Systems (GIS) layers to determine if high titers are concentrated around human developments, water sources, etc. Once potential disease areas are determined, we will be able to estimate the effect that the disease might have on local pronghorn populations. For instance, bluetongue virus has caused large-scale epizootics in the western United States, but BRSV has not been implicated in die-offs.

### **Methods for Objective 5**

In order to compare disease exposure to fawn recruitment, antibody titers from pronghorn in low recruitment sites will be compared to titers from pronghorn in high fawn recruitment sites for all diseases evaluated. The proportion of positive animals for each disease in each site will be used to determine statistical significance using appropriate statistical methods. Data will be analyzed by individual GMU and by pooling all animals in GMUs with high recruitment and pooling all animals from GMUs with low fawn recruitment.

## **COMPONENT 2-NUTRITION**

### **Background**

Pronghorn, being ruminants, are able to utilize a vast array of forage to obtain nourishment because gastrointestinal microbes are able to easily convert forage into usable nutrients (Wallach and Hoff 1982). Energy and protein requirements for adult ruminants vary with reproductive cycle, and late gestation and lactation require the highest protein and energy intakes for females (Nelson and Leege 1982). Energy requirements and food intake increase from 17 to 32 % in pregnant mammals whereas energy expenditure increases from 65 to 215 % in lactating females (Robbins 1993). During pregnancy, protein requirements increase 3 times over requirements for maintenance (Nelson and Leege 1982). As a result, the protein and energy intake needed to provide adequate nutriment to adult female pronghorn increases drastically during

spring and summer. If forage is deficient in either energy or protein during the fawning period, fawns could be born weak, or milk provided to fawns by females could lack adequate nutrients for proper fawn growth. Koerth et al. (1984) determined seasonal diets and quality of the diets for pronghorn in Texas and found that pronghorn primarily ate forbs year round, and forbs generally contain higher protein concentrations than other vegetation (Stephenson et al. 1985). Protein content in the overall diet varied from 9.8 % in winter to 11.4 % in spring. Authors compared this to predicted requirements for deer and concluded that year-round diets would meet pronghorn requirements for maintenance. Given that pronghorn does are in the third trimester of pregnancy in spring, it is unknown if 11.4 % protein would meet requirements of pregnant or lactating does, and Koerth et al. (1984) hypothesized that lack of adequate nutrition during spring could contribute to low fawn production in Texas.

Dunbar et al. (1999) measured nutritional blood parameters in pronghorn from Hart Mountain National Antelope Refuge in Oregon where the population of pronghorn had decreased 29 % from 1990 to 1995. In 1995, fawn to doe ratio dropped to 1 fawn per 100 does. Mean blood urea nitrogen levels for both adult females and fawns were significantly lower than those found in fawns and does from a healthy population in Alberta, and the authors attributed this difference to a low protein diet consumed by pronghorn in Oregon. Given the variable nature of precipitation in Arizona and that nutrient content varies in plants with season (Van Soest 1994), inadequate protein or energy content of forage during the spring and summer could contribute to poor fawn recruitment in Arizona.

Several nutritional deficiencies have been reported for pronghorn, but pronghorn requirements for most minerals remain unknown. Selenium (Se) deficiency has been reported in pronghorn from Idaho, and the deficiency coincided with decreased fawn recruitment and clinical signs of “weak calf syndrome” in newborn fawns (Stoszek et al. 1980). Flueck (1994) measured the effect of Se on reproduction of black-tailed deer (*Odocoileus hemionus columbianus*) in California and found that Se supplementation increased fawn production. Deer given Se increased fawn survival from 32 fawns per 100 does to 83 fawns per 100 does after supplementation. Dunbar et al. (1999) found that adult pronghorn from a site with chronically low fawn recruitment had a mean liver

selenium concentration below the minimum adequate level for domestic ruminants. Forage sampled from Arizona is deficient in Se (Kubota et al. 1967, Robbins 1993, Frederick 1997), and Heffelfinger et al. (1999) found that 73% (73/100) of liver samples from pronghorn around the state were below the 0.25 ppm minimum adequate level reported for domestic goats, cattle, and sheep (Puls 1995).

Copper (Cu) deficiency has also been documented in many free-ranging ungulates including pronghorn (Robbins 1993, Heffelfinger et al. 1999). Heffelfinger et al. (1999) analyzed liver tissue from 100 pronghorn around the state for copper concentration and found that 97% of the pronghorn had liver copper levels below the 25 ppm lower limit for domestic ruminants. During the same study, 99 pronghorn serum samples from Arizona were analyzed for copper concentration, and 82 (82.8%) were below adequate levels for domestic ruminants (0.70 ppm) for serum copper, with the average concentration being 0.59 ppm throughout the state (range=0.22-1.8 ppm).

Although data regarding mineral status and requirements for pronghorn are lacking in Arizona, it seems that pronghorn have levels of copper and selenium below those seen in healthy domestic animals. Given that copper and selenium deficiencies influence reproductive capabilities of domestic ruminants (Robbins 1993), it is certainly possible that mineral deficiency plays a role in low fawn recruitment in Arizona's pronghorn populations. In addition, deficiencies of both copper and selenium have caused clinical illness in domestic animals in Arizona (Bradley et al. 1997, Frederick 1997).

Given that energy and protein requirements increase dramatically for ruminants during late gestation and lactation (Nelson and Leege 1982), and that Arizona pronghorn have been shown to have low mineral levels in tissues, we believe that determining the protein, energy, and mineral content of pronghorn forage in Arizona will be of interest to wildlife managers.

## **Objectives**

1. Describe the diet composition of pronghorn populations at several sites in Arizona.
2. Evaluate the nutritional quality of those diets.

3. Evaluate the ability of those diets to meet the nutritional needs of the pronghorn populations.
4. Determine if pronghorn in sites with low fawn recruitment are on a lower plane of nutrition than pronghorn in areas of high fawn recruitment.

**Predictions and Research Hypotheses**

1. Pronghorn diets and the concomitant nutrients available to pronghorn on low recruitment sites will differ from those provided on high recruitment sites.
2. Pronghorn diets in most sites will lack adequate nutrients, primarily copper and selenium.
3. Does from low fawn recruitment sites will not be able to meet protein and energy requirements during late gestation and lactation.

**Methods for Objectives 1 - 4**

Field Methods

Data collection will occur over 4 time periods: late gestation, parturition, lactation, and prior to conception. Elevation on Anderson Mesa in GMU 5B varies between 6,700 and 7,200 feet (Neff and Woolsey 1979) and Garland Prairie in GMU 8 is approximately 6,800 feet. Pronghorn at this elevation are predicted to have a mean fawning date of late May and early June (7,000 feet is June 3; Ticer et al. 2000), however, biologists in the area believe that mid-May is more realistic (J. Goodwin, AGFD, personal communication). Pronghorn gestation averages 252 days (O’Gara 1978), and the last trimester would fall approximately on March 11. Migratory pronghorn generally return to Garland Prairie by April (AGFD, unpublished data), so collecting data during the third trimester will likely be feasible in April. Scenarios for the GMU 1, 2B, 34B, and 36B sites have also been determined and the approximate timeline for collection periods is below.

Time period	GMU 1	GMU 2B	GMU 36B	GMU 34B	GMU 5B	GMU 8
Late Gestation	4/25 – 5/10	4/25 – 5/10	3/20 – 4/5	4/1 – 4/15	4/10 – 4/25	4/1 – 4/15
Parturition	5/25 – 6/10	5/25 – 6/10	4/20 – 5/10	5/5 – 5/20	5/10 – 5/30	5/5 – 5/20
Lactation	7/5 – 7/20	7/5 – 7/20	6/5 – 6/25	6/15 – 6/30	6/20 – 7/10	6/15 – 6/30
Conception	8/20 – 9/10	8/20 – 9/10	8/1 – 8/15	8/10 – 8/25	8/20 – 9/10	8/15 – 8/30

#### Fecal Collection for Diet Analysis:

A group of pronghorn will be located and observed with a spotting scope until the majority of the individuals have defecated. A rangefinder will help determine distance to the group. Individual fecal pellet groups will be collected and labeled with date, number of pronghorn in the group, number of each sex in the group, GPS location, time period, a unique number for each pellet group, and site. Fecal pellets from individual animals will also be collected and pooled if necessary. The unique number for each pellet pile will be used to keep records on animals used in each composite sample. Five pellet groups from each study site for each time period will be pooled to create 1 composite sample for diet analysis. At least 4 composite samples will be analyzed per time period per site, so a minimum of 20 pellet groups will be collected from each study site during each of the 4 critical time periods identified. Fecal samples will be frozen until laboratory analyses are performed.

#### Plant Collection:

Samples of all known and suspected forage plant species in an area will be collected to create diets for nutritional analyses and to create reference slides for microhistological diet determination (see laboratory methods). Given that pronghorn are selective feeders, only palatable portions of plants (i.e. leaves and terminal buds of shrubs) will be collected (O' Gara 1978). Once a group of pronghorn have been sighted and feces collected, all plant species will be harvested from at least 3 areas adjacent to the fecal collection site. A larger sample will be taken from plants that pronghorn have been shown to eat regularly. At least 75 g (dry weight) of every plant species within a 50-m radius of the fecal collection site will be collected. Again, GPS locations of plant collection sites will be recorded.

#### Laboratory Methods

##### Microhistological Analysis:

Diet composition will be determined using microhistological analysis for each composite sample by site by season. The procedures described by Holt et al. (1991) will be used to process all fecal and reference materials. Briefly, relative density of each plant

species in fields of a microscope preparation of composite feces will be used to determine composite diets (Koerth et al. 1984). Microhistological analyses will be performed at the 90% ( $P=0.1$ ) confidence level.

#### Nutrient Analyses:

Nutrient analysis to estimate diet quality will be performed on the composite diet described above. A composite diet containing the proportions determined using microhistological analysis will be assembled and analyzed for each sample by site by season combination. Specific nutrients to be measured include: cell wall, cellulose, and hemicelluloses (Goering and Van Soest 1970), crude protein (micro-kjeldahl analysis), gross energy (bomb calorimetry), and mineral content (copper and selenium using absorption spectrophotometry). In addition to chemical analyses, composite diets will be evaluated for digestibility using *in vitro* digestion (Goering and Van Soest 1970) to determine protein and energy digestibility.

#### Data Analysis

This study component is designed to test 2 separate hypotheses. The first hypothesis is that there is a difference in species composition in pronghorn diets between high and low fawn recruitment sites. This hypothesis will be tested using a completely random three-factor factorial design, with study sites, season (late gestation, parturition, peak lactation, time of conception) and plant species as factors. These data will be analyzed using a one-way analysis of variance (ANOVA). If significant differences are detected, determination of the nature of the differences will be made using a LSD mean separation test (Zar 1999). Additionally, diet similarity will be described using the procedure of McArthur and Levins (1967).

The second hypothesis to be tested is that there is a difference in the diet quality between high and low fawn recruitment sites. The design for this portion of the study is a completely random two-factor factorial. The 2 factors are site and season. This analysis will be performed on each nutrient (protein, energy, fiber, and minerals) separately using a one-way ANOVA, and any significant differences further tested using a LSD mean separation test (Zar 1999). All statistical tests will be performed at the 90 % confidence ( $P = 0.1$ ) level.

In addition to determination of differences in diet composition and quality, each diet will be compared with the modeled expected needs of the pronghorn doe to determine adequacy of the diet to meet nutritional requirements at each stage of the reproductive cycle (Holt 1992, Miller 1980, Robbins 1993).

## **COMPONENT 3-WATER REQUIREMENTS**

### **Background**

Water is an essential nutrient needed to sustain metabolic processes, control body temperature, lubricate joints, and excrete wastes (Robbins 1993). In addition, milk of many ungulates at mid-lactation is comprised of between 70 and 85 % water, making water even more precious during lactation (Robbins 1993). Water can be acquired in 3 ways: 1) through drinking free water, or that found in lakes, streams, drinkers, etc., 2) through metabolic processes, or oxidation of hydrogen-containing compounds, and 3) through food items, or preformed water.

Several studies have investigated the importance of water for pronghorn, but certain results are equivocal. Clemente et al. (1995) found that radiomarked pronghorn in the Chihuahuan Desert in southern New Mexico stayed within 3 km of livestock drinking tanks. In addition, pronghorn densities in the Red Desert of Wyoming were higher in areas where free water was available in drinking troughs than in areas without free water. Once water in the troughs was turned off, distribution of pronghorn did not change. However, given that water content of forage was high throughout the experimental period, preformed water may have been adequate to fulfill water requirements (Deblinger and Alldredge 1991). Sundstrom (1968) found that pronghorn densities were much higher in areas that contained free water than in areas without water; 85 % of the pronghorn in the study area were located in areas that contained 90 % of the free water on the site. Beale and Smith (1970) measured forage use, water consumption, and fawn production of pronghorn in western Utah and found that pronghorn drank from sources of free water only when succulent forage species, particularly forbs, were not available. As a result, they concluded that pronghorn were able to acquire enough water through food

sources to meet their needs during part of the year. In addition, fawn production was positively correlated with precipitation received during the previous summer.

Fox (1997) hypothesized that Sonoran pronghorn in Arizona are able to acquire adequate water through succulent forage during most of the year, but does may not acquire adequate preformed water during lactation. Moreover, Ockenfels et al. (1992) located pronghorn fawns < 1 km from an identified water source during the first 6 months, potentially due to the increased water requirements for does during lactation. Perhaps female pronghorn in Arizona need to drink free water during lactation, but can acquire adequate preformed water during the remainder of the year. Pronghorn in the western United States have adapted several mechanisms to conserve water, including decreased water content of urine, decreased respiratory rate, and cessation of panting (Yoakum 1994). Despite these modifications, pronghorn population densities were still highest in areas where free water is available; Yoakum (1994) concluded that pronghorn densities in dry desert environments were <1 per square mile, whereas densities in Wyoming and Montana, where free water was available, averaged between 5 and 10 pronghorn per square mile.

Pronghorn water deprivation studies showed that animals lost weight, rested more often, and became listless when deprived of water, but once animals were offered water, they drank readily and regained weight immediately (Beale and Smith 1970, Wesley et al. 1970). Both availability and quality of water are important to pronghorn. In Arizona, Hervert (2001) documented pronghorn using man-made water sources, but quality of the water is a factor. Pronghorn avoided water that contained >5000 ppm total dissolved solids (O’Gara and Yoakum 1992). Lee et al. (1998) concluded that both pH and dissolved solids in water influence pronghorn use of water sources; pronghorn avoided water with a pH higher than 9.2 (Sundstrom 1968).

Apparently pronghorn in Arizona drink free water when it is available, especially during periods when succulent forages, particularly forbs, are not available. In addition, lactating does have an increased water requirement, and may require free water to meet demands when fawns are suckling. Given that, it seems that pronghorn populations remain healthier and could have higher fawn survival when they have access to free water.

## **Objectives**

1. Locate sources of free water in each study site and GPS locations for use in GIS database.
2. Determine water availability at each water source during periods when pronghorn are in the third trimester of pregnancy, after parturition, during lactation, and just prior to conception.
3. Determine water quality at sites where water is available.
4. Determine if availability and quality of free water differs between sites of high fawn recruitment and low fawn recruitment.

## **Approach**

Sources of free water will be identified using data, maps, and records from the United States Forest Service (USFS), United States Fish and Wildlife Service (USFWS), AGFD, and State of Arizona Land Department (SLD). AGFD personnel will also identify water sources on each site as part of routine field excursions. For each water source, we will determine when water is present, quality of the water at the site, and if free-ranging pronghorn would have access to the water. We will compare water availability and water quality by site and by fawn recruitment status.

## **Predictions and Research Hypotheses**

1. Free water will be available to pronghorn at all sites, but number of sources and the water quality will differ among sites.
2. Pronghorn in sites with high fawn recruitment will have better access to high-quality water sources than pronghorn in low recruitment sites, especially during the summer lactation period.

## **Methods for Objective 1**

Three available GIS cover layers were used to initially identify sources of free water in each study site. AGFD personnel created a water development cover layer that mapped locations of all man-made developments constructed by AGFD, and SLD digitized all naturally occurring springs in Arizona and a cover layer was created from those locations. In addition, a layer identifying all perennial water sources (i.e. lakes, streams) in Arizona was created and was used to determine sources of free water in each site. Using the 3 GIS cover layers, maps of potential sources of free water were created

for each study site (Fig. 2 *a-f*). Now that preliminary maps of tanks, perennial water sources, and natural springs have been created, regional AGFD personnel will be asked to verify locations of water sources. Additional sources will be added, including those located during fieldwork. We will visit each water source and record a GPS location. We will then create 1-km buffer zones around each water source to determine the percentage of each study site that is 1-km from a water source.

#### **Methods for Objectives 2-4**

Once water sources have been located, we will visit at least 15 sources in the core of each study site to evaluate pronghorn access to the water at each site (i.e., do fences enclose the source?). All sites with adequate access will be monitored every month from April through August (late gestation through conception for pronghorn in Arizona) for the presence of water. AGFD personnel will visit sites on the ground, or if possible, we will fly over water sources to determine if water is present at the identified source. Water samples will be collected from at least 10 sources in the core of each study area each month for water quality analyses (Rosenstock and Rabe 2000). Given that pronghorn have been shown to avoid water sources with high dissolved solids and high pH (Sundstrom 1968, O’Gara and Yoakum 1992), water samples will be analyzed for total dissolved solids, pH, and salinity. We will then determine if water sources from sites with low fawn recruitment differ in number or quality of water sources from sites with high fawn recruitment using appropriate statistical techniques. Lastly, we will determine seasonal trends in water availability and water quality by site in order to compare water availability with the identified critical periods for pronghorn does.

### **COMPONENT 4-PREDATOR INFLUENCE ON FAWN RECRUITMENT**

#### **Background**

Low fawn recruitment has been implicated as a major contributing factor to pronghorn decline in Arizona, and Neff (1986) identified low recruitment as the most important management issue for pronghorn in Arizona. What causes low fawn recruitment? High fawn mortality has been well documented for pronghorn, with between 25 and 85 % of fawns perishing during the first 6 months of life in radio-

telemetry studies (Vriend and Barrett 1987, Ockenfels et al. 1992, Rothchild et al. 1994). Gregg et al. (1999) found that 83 % of 104 radiomarked pronghorn fawns died during a 2-year study on Hart Mountain National Antelope Refuge in Oregon. Predation accounted for 72 % of the deaths, with coyotes (*Canis latrans*) being the primary predator during the first 3 weeks after birth. Trainer et al. (1983) found that coyote predation accounted for approximately 60 % of fawn mortality on study areas in southeastern Oregon during 2 summers, and Barrett (1984) studied pronghorn fawn mortality in Alberta and concluded that predation was the primary cause of death (68 %), with coyotes being the main predators responsible.

Neff et al. (1985) reviewed literature regarding predator control and fawn recruitment for pronghorn and white-tailed deer (*Odocoileus virginianus*), and they concluded that fawn recruitment for both species can be increased through coyote control measures. In addition, studies on Anderson Mesa, Arizona found that pronghorn fawn survival rates increased after coyote control measures were initiated, but increased fawn survival lasted for only 2 years after a removal event. Neff and Woolsey (1979) conducted a fawn survival study on Anderson Mesa and concluded that coyote predation was the primary cause of fawn mortality. Fawn survival increased dramatically after coyote control measures were established, and lack of adequate hiding cover for fawns was identified as a contributing factor in high coyote predation. In a follow-up investigation, Neff et al. (1985) modeled effects of coyote control on fawn recruitment on Anderson Mesa. They concluded that the pronghorn herd would increase most drastically when coyote control was implemented in 3 of 5 years.

In order to determine if coyote predation influences fawn recruitment on our study sites, we will compare relative predator abundances on 6 study sites. Biologists have attempted to identify adequate methodology for estimation of predator numbers for decades (Wood 1959, Warrick and Harris 2001), but a universally accepted protocol has not been established. Howling responses, aerial counts, scat deposition rates, track counts, and scent station visitation rates have all been proposed as methods for carnivore abundance estimation, but each method has specific assumptions and biases that must be considered (Knowlton 1984). Knowlton (1984) reviewed methodology for determining relative abundance of coyotes, and he concluded that 2 methods could be useful when

attempting to estimate abundance on small study areas: the scat deposition method and the scent station visitation method. The scent station method requires researchers to construct many scent stations that consist of a scent attractant in the center of a circle of fine sand 3 ft in diameter (Roughton 1979). Scent stations are constructed every 0.3 miles along unimproved roads and as many as 50 stations have been used. Stations are checked for animal tracks each day for 4 days. Scent stations are useful under strict conditions with rather large study sites, methods are very labor intensive, and a trained observer must identify tracks around scent stations (Roughton 1979). The scat deposition method requires little modification of the environment, and untrained personnel can walk transects to find and collect scats (Knowlton 1984). The method requires personnel to walk a series of transects (from 0.25 to 1.0 mile) on trails or dirt roads and clear predator scats from the roads. Transects are walked in each direction to ensure that all scats have been removed. After 5 to 15 days, transects are re-walked in each direction and scats are again collected. Estimates are given as number of scats/mile/day (Knowlton 1984). Due to ease of use, we will implement the scat deposition method to estimate relative abundances among sites.

### **Objectives**

1. Determine relative predator abundances for the 6 study sites during peak pronghorn fawning periods and during peak lactation using the methods of Knowlton (1984) and Cunningham and Kirkendall (in review).
2. Compare predator indices for low pronghorn fawn recruitment sites with indices in high recruitment sites.

### **Approach**

In order to compare predator densities in high fawn recruitment sites to those in low recruitment sites, we will clear predator scats from roads, game trails, and hiking trails in each site and later re-walk roads and trails to count the number of predator scats on the site. We will then use number of days since the trails were cleared of scat to determine scats deposited/mile/day. These statistics will be compared by site and by fawn recruitment status.

## **Predictions and Research Hypotheses**

1. During peak fawning time, predator sign will be more abundant on sites that suffer from low fawn recruitment than on sites with high fawn recruitment.
2. On all sites, pronghorn recruitment will be highest in years when predator estimates are lowest.

## **Methods for Objective 1 and 2**

Scat deposition rates will be measured during peak fawning periods for each field site, and this period will differ slightly by site, reducing the likelihood of experiencing a shortfall in personnel. We will estimate predator abundance by using a set of permanent scat transects arranged on hiking trails and unmaintained roads (Fig. 3 *a-f*). A GPS location will be taken at the beginning of each transect. We will walk each transect in both directions to clear the trail of scats, and then we will re-walk each transect 15 days later and collect scats. Number and length of transects will vary with site, but a minimum of 2.5 miles will be traveled (10 transects, 0.25-mi. each). Fox scats will be distinguished from coyote scats with the methods of Danner and Dodd (1982). An index of predator abundance will be calculated for each site using the following equation:

$$\{(\# \text{ of scats from species } x)/(\# \text{ of nights of scat accumulation})\} \times 100.$$

The indices will be compared by study site and by fawn recruitment status using appropriate statistical tests. We will also compare fawn recruitment each year to the predator estimation index from the same year via correlation analysis to determine if predator presence correlates with fawn recruitment on all sites.

## **COMPONENT 5-FAWN HIDING COVER**

### **Background**

Pronghorn use the “hider” strategy of predator avoidance, where young are concealed for several weeks after parturition (Alldredge et al. 1991); pronghorn fawns spend approximately 90 % of their time bedded during the neonatal period, or first 3

weeks of life (Barrett 1981). Ticer and Miller (1994) speculated that neonatal fawns use concealment and predator detection to escape predation, whereas fawns older than 3 weeks use the same detection and flight response as adults. It is during the first 3 weeks of life, while fawns are primarily bedded, that the majority of pronghorn fawn mortality due to predation occurs (Neff and Woolsey 1979; Autenrieth 1982). During this time, does remain separated from fawns for periods of 1 to 6 hours (O' Gara 1978). Thus, does provide fawns with minimal help in predator detection and avoidance (O'Gara 1978). Prior to giving birth, pronghorn does choose parturition sites and remain in these sites for several hours before and after birth (O'Gara 1978). In order to avoid predators, pronghorn does hide newborn fawns in available vegetation and consume excrement from fawns to decrease scent at fawn bed sites (O'Gara 1978). After the first few hours, fawns select bed sites, but does continue to consume feces and urine produced by fawns. Fawns also blend into the surrounding vegetation and lack scent glands early in life, thereby decreasing detectability by predators (Alldredge et al. 1991). Neonatal fawns normally rest with their heads up, but once movement is detected, the main predator avoidance tactic is to lie still with their eyes open and ears lowered (O'Gara 1978). Given that neonatal fawns do not run from predators, it is imperative for fawns to be concealed by adequate vegetative cover to hinder search efforts by carnivores. In addition, fawns must detect potential predators prior to changing positions within bed sites.

Several investigations of pronghorn fawn bedding sites have been conducted, but exact characteristics of bed sites seem to vary with location. In Idaho, pronghorn fawn recruitment was positively correlated with fawn hiding cover during the first 3 weeks of life (Autenrieth 1982), and increased cover at fawn bed sites was associated with higher temperatures as well (Autenrieth 1984). Autenrieth (1984) reported a correlation between percent total cover at 760 fawn bed sites and fawn recruitment numbers. Bodie (1978) studied mortality of fawns in Idaho and found that predators killed neonatal pronghorn fawns from tall sage/foothill habitat more often than fawns from the short sage/grass community type. He speculated that does could better detect predators and therefore defend fawns in short sage environments. Alldredge et al. (1991) measured vegetation characteristics at fawn bed sites in Wyoming and found that fawns chose sites

with more shrub canopy cover when compared to random sites within a fawn home range. Alldredge et al. (1991) speculated that fawns chose sites that provided adequate thermal cover and concealment, while lacking visual obstruction. Barrett (1981) measured fawn bed site characteristics in Alberta and found that grass and forbs over 25 cm tall, small depressions in the ground, and bare patches of ground were important for fawn bed site selection. In addition, fawns initially located in heavy cover were more likely to be re-located than fawns found in areas with little cover, suggesting that cover helped conceal fawns from potential predators. Tucker and Garner (1983) measured height of vegetation at fawn bed sites and at adjacent sites and found that fawns < 4 weeks old chose bed sites with taller vegetation than nearby areas.

In central Arizona, Ticer and Miller (1994) measured characteristics of 111 pronghorn fawn bed sites and found that fawns selected areas with a mean grass height of 29 cm and a mean forb height of 12 cm. Fawns avoided areas with high shrub densities, but when present, mean shrub height differed with age of fawn; those younger than 3 weeks chose sites with taller plants than fawns older than 3 weeks. It was thought that fawns rely upon camouflage and detection of predators until they are 3 weeks old, but employ “see and flee” predator avoidance tactics once they are old enough to travel with does. Therefore, fawns seemed to choose bed sites that would allow for adequate predator detection while providing cover for camouflage. Ticer (1998) found that fawns avoided sites with tall grass (> 15 cm) and concluded that fawns were selecting sites that allowed for early predator detection.

Results from various bed site structure and cover investigations are difficult to interpret, but it seems that both visual obstruction and vegetative cover are important when determining appropriate fawn bed site characteristics in an area.

Fawning areas and subsequent fawn bed sites are often located near water, potentially due to increased water intake requirements of lactating does (Beale and Holmgren 1974, Ockenfels et al. 1992, Ticer and Miller 1994). Ticer and Miller (1994) found that fawns < 3 weeks of age selected bedding sites within 800 m of a water source, and Ockenfels et al. (1992) found that neonatal fawns had very small home ranges (approximately 1 km<sup>2</sup>) that were located < 1 km from water sources. Barrett (1978) noted that fawns < 1 week of age were often bedded near intermittent water sources as

well. Given their proximity to water, we will focus on evaluating potential fawn bed site habitat near water sources for visual obstruction and cover in our 6 study areas.

### **Objectives**

1. Determine overall cover in potential pronghorn bedding sites in 6 study areas.
2. Determine visual obstruction in potential pronghorn bedding sites in 6 study areas.
3. Determine if sites with high fawn recruitment differ from sites with low recruitment in regard to cover and visual obstruction at potential pronghorn fawn bedding areas.

### **Approach**

We will compare cover and visual obstruction measurements at potential fawn bedding sites with fawn recruitment status in each study area. Measurements will be taken when fawns in that study area would be between 1 day and 3 weeks old, given mean fawning date for the area (Ticer et al. 2000). Due to proximity of bed sites to water, cover and visual obstruction data will be gathered within 1 km of 10 sources of free water in each study area (Ockenfels et al. 1992, Ticer and Miller 1994).

### **Prediction and Research Hypothesis**

1. Visual obstruction will be lower and small shrub and grass cover will be higher in areas with high fawn recruitment than in areas of low fawn recruitment.

### **Methods for Objectives 1 and 3**

Canopy cover will be determined at 10 random points at each of 10 water sources in the core of each study area. Random UTM coordinates from within a 1-km buffer zone around waters will be used to determine sites for data collection. Cover will be measured using a modified line-intercept method (Higgins et al. 1994, Bristow and Ockenfels 2000). Briefly, a 25-m rope will be marked at every 1-m interval. Vegetation at each interval will be evaluated to determine if it is 0 to 15 cm tall, 15 to 30 cm tall, 30 to 45 cm tall, or taller than 45 cm. The direction of the tape will be determined from a list of random degrees and 4 transects, at right angles to each other, will be evaluated. Percent cover will be measured by determining the percentage of marks on the rope

covered by vertical projections of vegetation. Percent cover from the 4 transects will be summed to get a percent cover at each site. Averages from all 10 water sources will be used to evaluate cover by study area and by fawn recruitment status using appropriate statistical methods.

### **Methods for Objectives 2 and 3**

Visual obstruction data will be collected with a modified visibility board (Bristow and Ockenfels 2000). A 50-cm by 50-cm visibility board marked at 10-cm intervals will be placed at 10 random sites around each water source and an observer will walk 4 m in a random direction to view the board (Higgins et al. 1994). We will view the board through a PVC joint at 1-m height. The percentage of each interval covered by vegetation will be noted and used to compare visual obstruction at every height interval. We will then compare visual obstruction at 10, 20, 30, 40, and 50 cm intervals to determine if obstruction varies by site or by interval. We will then compare those mean obstruction numbers to fawn recruitment status by site using appropriate statistical methods.

## **COMPONENT 6-RECREATIONAL USE**

### **Background**

Behavior patterns of wildlife species are often influenced by recreational use of habitat by humans, and the impact of recreational use on pronghorn varies with traffic volume (Lee et al. 1998). Currently, the impact that recreational use of roads in pronghorn habitat has on pronghorn populations in Arizona is unknown, but potential behavioral changes could cause does to neglect fawns during lactation. In addition, vehicle traffic could force pronghorn out of optimal habitat with prime forage, thereby influencing nutrition of doe and fawn during critical time periods. This portion of the study is designed to measure relative use of roads in pronghorn habitat during critical life stages for pronghorn.

Few studies have investigated impacts of human disturbance on pronghorn, but Segerstrom (1982) investigated the effect of coal strip mine on antelope populations in Wyoming. In particular, he determined how presence of heavy machinery, light vehicles, humans, and trains influenced pronghorn behavior, and he rated these behavioral changes

by energy needed to carry out each response. For instance, lack of reaction to disturbance was rated 1 and rapid, immediate flight was rated at 10. He found that pronghorn remained further from light vehicles and humans on foot than random points associated with these disturbances. In addition, pronghorn stayed furthest away from light vehicles. Airplanes, vehicles, and humans on motorcycles elicited the strongest behavioral responses by pronghorn as well. Apparently, vehicular disturbance causes pronghorn to change behavior dramatically.

HOBO<sup>®</sup> (Onset Computer Corporation) units are small, computerized data loggers that can measure when motorized devices change between on and off, using the current emitted and vibrations as cues to log information. The data loggers have magnets attached so they can adhere to metal surfaces easily. They record time and date for each event, can store up to 2,000 events, and information can be downloaded into a laptop computer in the field. These units have been used by AGFD personnel in region II to successfully measure recreational use of roads in the region (R. Miller, AGFD pers. communication). The data loggers are relatively inexpensive and very convenient to use. As a result, our goal is to determine a relative index of recreational use using several HOBO<sup>®</sup> units affixed to cattle guards in each site.

### **Objectives**

1. Determine the relative recreational use by motorized vehicles on each study site using HOBO<sup>®</sup> units.
2. Determine if sites with high fawn recruitment are exposed to higher recreational pressures than sites with low fawn recruitment.

### **Approach**

HOBO<sup>®</sup> units will be placed on at least 5 cattle guards in each site. Cattle guards will be chosen such that recreational use on the majority of the site will be recorded by at least one of the HOBO<sup>®</sup> units. Data will be downloaded into a laptop computer every 2 months and the number of motorized vehicles crossing each cattle guard during each critical period will be tallied.

### **Prediction and Research Hypotheses**

1. On all sites, recreational use as measured by motorized vehicle traffic over cattle guards will vary with day of the week and time of year.

2. Study sites with low fawn recruitment will have increased numbers of motorized vehicles in the area during critical periods for pronghorn when compared to sites with high fawn recruitment.

### **Methods for Objectives 1 and 2**

HOBO<sup>®</sup> units will be affixed to at least 5 cattle guards near the core of each site in order to record passage of motorized vehicles in each site. The time and date of each event will be recorded by HOBOS<sup>®</sup> units and data will be downloaded into a laptop computer in the field using BoxCar Pro 4.0 software. Data will be summarized by day of the week and time of year, with special interest in periods of late gestation, parturition, lactation and conception for pronghorn in each site. The number of vehicles that pass per day will be calculated by critical time period for each site, and the data will be compared to fawn recruitment to determine if recreational use in sites with high fawn recruitment varies from use in sites with low recruitment.

### **COMPONENT 7-SHRUB AND TREE DENSITY**

#### **Background**

Adult pronghorn are able to run at speeds of up to 60-70 km an hour, and average cruising speed is near 40 km per hour (Yoakum 1978). Given their ability to outrun potential predators, pronghorn rely upon early predator detection and fleeing as a survival strategy. Habitats containing vegetation that obscures vision for pronghorn are therefore less preferred than those that allow for early predator detection.

Pronghorn habitat has been well studied over the last 10 years (Yoakum 1978, Ockenfels et al. 1994, and others), and pronghorn generally prefer to inhabit grassland/shrubland habitat (O’Gara 1978). Lee et al. (1998) identified optimal biotic habitat conditions for pronghorn as grasslands with <10 % shrub cover or shrub/steppe environments with between 10 and 35 % shrub cover. Between 5 and 10 species of shrubs were often present as well. Tree diversity and density were low with 2 or fewer tree species being found in pronghorn habitat. Tree density in good pronghorn habitat was 5 per hectare (Alexander and Ockenfels 1994). Low vegetation heights (25 to 46 cm) were preferred and heights of 63 cm or higher were avoided. Yoakum (1978) summarized findings on habitat requirements for pronghorn. Vegetation on pronghorn

habitat averaged 38 cm in height and pronghorn avoided areas where vegetation was higher than 61 cm. Alexander and Ockenfels (1994) investigated pronghorn habitat use of juniper stands in central Arizona and found that tree density was negatively correlated with pronghorn use. The high use areas for pronghorn contained an average of 4.7 trees per hectare, while non-use areas contained 155 juniper trees per hectare. Yoakum (1978) also stated that pronghorn rarely inhabit areas with ponderosa pine or juniper stands as well.

### **Objectives**

1. Determine if relative densities of shrubs and trees from 0-20 cm tall, 20-40 cm tall, 40-60 cm tall and those taller than 60 cm differ among sites.
2. Determine if relative tree and shrub diversity differs among sites.
3. Determine if fawn recruitment differs with relative density of vegetation in any of the height classes in objective 1.
4. Determine if fawn recruitment differs with relative diversity of trees and shrubs in the height classes in objective 1.

### **Approach**

We will measure relative densities of trees and shrubs at heights of 0-20 cm, 20-40 cm, 40-60 cm, and > 60 cm on study sites in order to determine whether taller vegetation influences pronghorn fawn recruitment. We will use a set of random points on each site to choose sampling areas. At each site, we will select a random compass bearing and determine the canopy cover and species of trees and shrubs in each height interval that intercept a 50 m line transect (Higgins et al. 1994). We will determine canopy cover every 2 m on the rope. We will measure vegetation characteristics in 50 m lines 90, 180, and 360 degrees from the original compass bearing, thusly measuring canopy cover at 100 total observations at each random point. We will use the mean number of trees and shrubs in each height interval on each site to measure the effects of relative tree and shrub densities on fawn recruitment. The average species richness by site will be used to determine if tree and shrub richness influences fawn recruitment.

### **Prediction and Research Hypotheses**

1. Relative density of vegetation 0-20 cm in height, 20-40 cm in height, 40-60 cm in height, and > 60 cm in height will differ among sites.

2. Diversity of trees and shrubs in each size class will differ among sites.
3. Fawn recruitment will be higher in sites with fewer trees and shrubs taller than 60 cm.
4. Fawn recruitment will be higher on sites that have few species of trees and shrubs.

#### **Methods for Objectives 1 - 4**

A minimum of 15 randomly selected sampling areas (UTM coordinates) per year will be evaluated in each study site. We will locate each random point by finding coordinates with a GPS unit. A tape will be laid out 50 m in the direction of a random compass bearing, and all trees and shrubs that intersect the line will be identified to species (Higgins et al. 1994). The height of the tree or shrub (0-20 cm, 20-40 cm, 40-60 cm, and > 60 cm) will be measured with a pole labeled at each interval. The number of trees and shrubs in each size class will be added to determine a number per sampling area and the numbers will be averaged for each study site. We will determine whether fawn recruitment is correlated with mean number of trees and shrubs in each size class or with mean species richness for trees and shrubs in each class.

### **COMPONENT 8-FENCE DENSITY AND STRUCTURE**

#### **Background**

Fences restrict the movements of pronghorn (Ockenfels 1994 and references therein), often preventing herds from moving into higher quality habitat (Hailey and DeArment 1972). White (1969) reported on a severe weather event that occurred in northern Arizona in 1967 where 94 inches (239 cm) of snow fell on Flagstaff in 1 week, covering most available forage for pronghorn. Low temperatures and low-lying fog also exasperated the problem. As a result, herds began to move out of the area but some individuals were trapped by six strand barbwire fences in higher elevational areas. A similar situation occurred in Wyoming during winter of 1971-72, where approximately 60 % of the pronghorn herd perished after a snowstorm (Riddle and Oakley 1973). Fence lines along Interstate 80 restricted pronghorn movements, and therefore both severe weather and restricted range contributed to high losses. Ockenfels et al. (1997) investigated effects of roads and fences on pronghorn movements in Arizona. Thirty-

seven collared pronghorn were monitored for two years (1,671 locations) and several animals crossed fenceless roads, but pronghorn did not cross fenced roads. In addition, roads and railroads with fences seemed to limit home ranges of collared pronghorn. Pronghorn rarely jump over fences, but will crawl under them (Spillett et al. 1967, Hailey and DeArment 1972, Lee et al. 1998). As a result, management strategies to improve fence structure to allow pronghorn passage have been identified (Spillett et al. 1967, Lee et al. 1998). Smooth-wire fences are best for pronghorn movement, but when barbed wire is needed, fences should have 3 strands of wire with the bottom strand consisting of smooth wire 41 to 46 cm above the ground. The top wire should not be taller than 91 cm. Moreover, new fences should have white rag flagging tied to the wire periodically to increase visibility of the fence for pronghorn.

The goal of this component is to determine fence structure and density on study sites and identify if population trends are correlated with the density of fences.

### **Objectives**

1. Determine the types of fences contained in each study area.
2. Determine fence density on each study area.
3. Determine if fawn recruitment differs with fence density and structure on 6 study areas.

### **Approach**

We will determine fence density by using maps of current fences in each study as guides. In addition, fences will be evaluated opportunistically in the field for several years. We will also fly over study sites to locate fences for evaluation. Fence density and structure will be determined for each site and relative indices for these measurements will be used to compare fence structure and density with fawn recruitment on each site.

### **Prediction and Research Hypotheses**

1. Fences will be common on all sites but structure will differ by site.
2. Density of fences that potentially confine pronghorn will be greater on sites with low fawn recruitment.
3. Pronghorn-friendly fencing will be more abundant on sites with high fawn recruitment.

### **Methods for Objectives 1, 2, and 3**

We will visit each fence in the site and describe fence structure using length of fence, height of each strand of fence, type of material used to construct the fence, and whether pronghorn could potentially get beyond each fence (i.e., is it pronghorn friendly?). Fence segments will be categorized using Spillett et al. (1967) and each fence type will be given a rating as to its ability to confine pronghorn. Fence density will be calculated using the overall kilometers (miles) of fence contained in each site and overall area of each site. In addition, density will be separated into categories for fencing that pronghorn cannot negotiate and for pronghorn-friendly fencing. Therefore, fence density will be calculated by fence rating. The density of fences that pronghorn can and cannot negotiate will be compared to fawn recruitment for each site.

### **COMPONENT 9-SOIL HEALTH**

#### **Background**

Mineral deficiencies have contributed to low recruitment of young in ungulate populations (Component 2, Stoszek et al. 1980, Flueck 1994, O'Hara et al. 2001), and mineral supplementation has been shown to increase conception rates of cattle in Arizona (Sprinkle et al. 2000). White muscle disease or muscular dystrophy is caused by lack of selenium in diet (Robbins 1993). Forage plants from several sites in Arizona contain lower than adequate levels of selenium, and it is likely that other minerals, such as copper and zinc are below adequate levels as well (Frederick 1997). Soil chemistry and type vary across geological area, with underlying bedrock dictating much of the soil chemistry (Kubota et al. 1967, Reilly 1996). For instance, plants found in clay soils often contain higher levels of selenium than plants in sand because clays retain selenium better, thereby providing increased selenium to plants (Gissel-Nielsen 1976). The pH of soil, levels of other minerals, such as phosphorous, nitrogen, and sulfur in soil, and the amount of organic matter in soil also influence selenium uptake by plants (Gissel-Nielsen 1976). Kubota et al. (1967) discussed the occurrence of white muscle disease in livestock and the selenium levels in plants and underlying parent soil material and found that disease was correlated to sites with low selenium in the soil; plants from areas with white muscle disease contained lower than adequate selenium. Soils from Arizona were identified as potentially selenium-deficient, and forage plants from Arizona were deficient as well.

Therefore, white muscle disease is a potential problem in the state. Moreover, alkaline soils in Arizona should provide selenium to plants if adequate selenium was present. Given the tie between soil chemistry, nutrient content in plants, and animal health, we will measure basic soil metrics and determine if patterns emerge when compared to fawn recruitment in the 6 study areas.

### **Objectives**

1. Measure Se, Cu, P, K, N, Mg, Ca, S, Fe, Mn, Na, Cl, and Zn levels in soils from 6 study areas.
2. Measure the amount of organic matter in soils from 6 study areas.
3. Determine if correlations exist between mineral levels in plants and soils in 6 study areas.
4. Determine if fawn recruitment differs with mineral level or organic matter in the soil.

### **Approach**

We will collect 4 soil samples from each study site in August of each year, using pronghorn locations from the summer to determine sites. Soil characteristics do not change significantly over months (Stu Buck, pers. comm., Dr. Steven Hart, Northern Arizona University, pers. comm.), so collecting samples in August should indicate overall soil conditions from April through August. Each sample will consist of 5 homogenized subsamples from aggregates of individual pronghorn locations. Samples will be analyzed for mineral and organic content at Spectron Labs, 34102 N. 12<sup>th</sup> Street, Phoenix, Arizona. Mineral levels and organic content of soils will be compared to fawn recruitment in each study site, and to plants collected in each site as part of the Nutrition Component.

### **Prediction and Research Hypotheses**

1. Mineral levels and organic content in soils will vary by site.
2. Mineral levels in plants will correlate to mineral levels in soil.
3. Selenium, copper, zinc, and organic matter will be higher in soils from high recruitment sites than in soils from low recruitment sites.

## **Methods for Objectives 1 - 4**

From April through August each year, we will be locating pronghorn and collecting fresh fecal pellets and associated plants in the area. A GPS location is taken each time a fresh fecal pellet pile is collected. Pronghorn are not randomly located in each study site. To the contrary, groups of pronghorn are often located in the same general area for several days or weeks. GPS locations from these samples will be mapped and we will determine appropriate soil collection areas from these pronghorn locations. Four soil sampling areas will be delineated and 5 subsamples of soil will be collected from each soil sampling site. The humus will be brushed away and the top 6 inches of soil will be collected and placed in a 1-liter receptacle. A total of 5 subsamples will be combined to create 1 sample from the study site. The collection protocol will be followed in 4 soil collecting areas per study site. Samples will be delivered to Spectron Labs in Phoenix and minerals and organic matter will be measured with Wakely-Black method. The soil sample will be digested in acid prior to being read for organics via spectrophotometry. All minerals except N and P will be measured after digestion in acid with an atomic absorption spectrophotometer. Nitrogen and P will be measured colorimetrically on a UV VIS spectrophotometer. Mineral levels and organic matter will be compared to levels in plants and to fawn recruitment in each study site. Appropriate statistical tests (correlation coefficients) will be calculated to determine trends.

## **GENERAL TIMELINE AND TECHNICIAN REQUIREMENTS**

During the first field season, we will collect all data on all components and we will evaluate sample size to determine if we can possibly gain valuable knowledge with the current methods, technician time, and sample sizes. We will then determine which components should continue and which should conclude after only 1 or 2 field seasons. Time schedules for all 4 years are outlined in Tables 1 – 4.

Technician requirements are outlined in Tables 5 – 11. The ideal situation is for each pair of sites to have 1 technician, so optimally we will have 3 technicians working from April through August each year.

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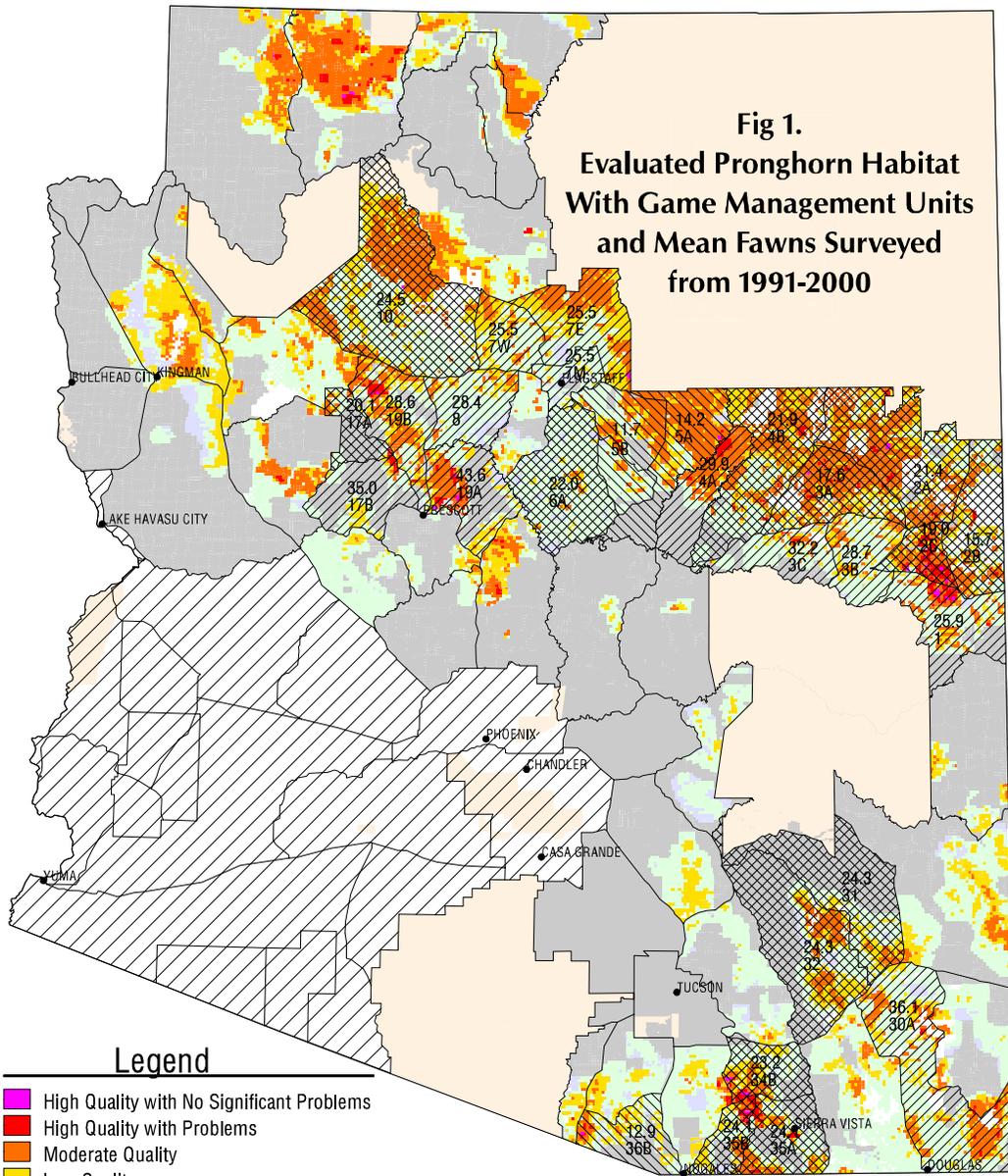
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**Fig 1.  
Evaluated Pronghorn Habitat  
With Game Management Units  
and Mean Fawns Surveyed  
from 1991-2000**



**Legend**

- High Quality with No Significant Problems
- High Quality with Problems
- Moderate Quality
- Low Quality
- Poor Quality
- Evaluated as Unsuitable
- Mapped as Poor or Unsuitable-Not Evaluated
- Access denied; not evaluated
- Tribal Lands
- Sonoran Pronghorn Range-Not Evaluated
- 0 - 15 Fawns
- 15 - 25 Fawns
- 25 or more Fawns



# Game Management Unit 2b

- Legend**
- Township/Range
  - Unit Boundary
  - Roads
  - Streams
  - Springs
  - AGFD Water Developments

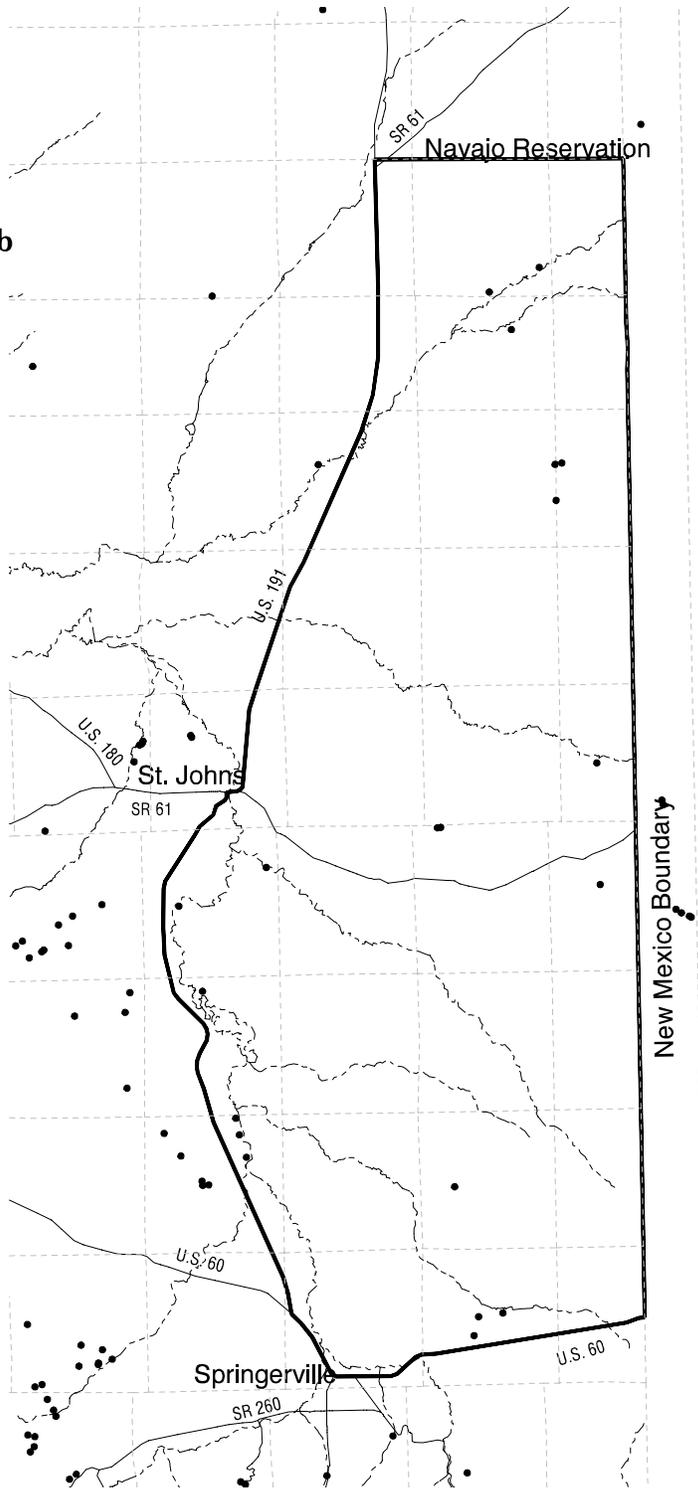
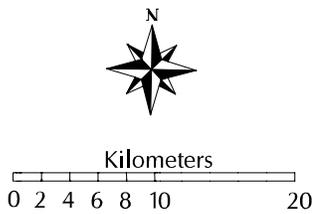
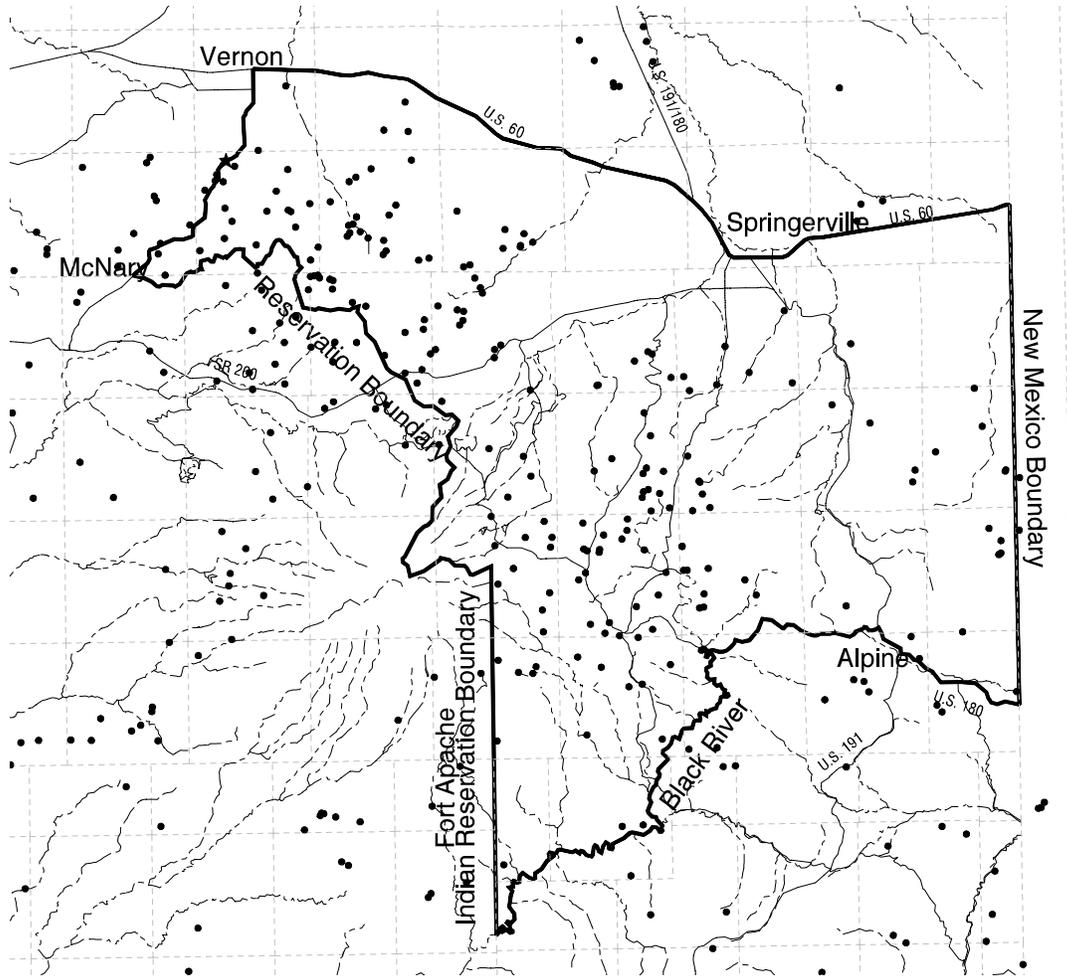


Figure 2 a. Water sources in GMU 2b



**Legend**

- ⚡ Township/Range
- ▬ Unit Boundary
- ▬ Roads
- ▬ Streams
- Springs
- ★ AGFD Water Developments

**Game Management Unit 1**

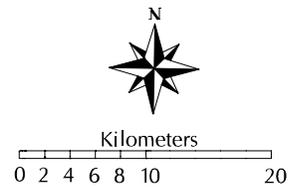
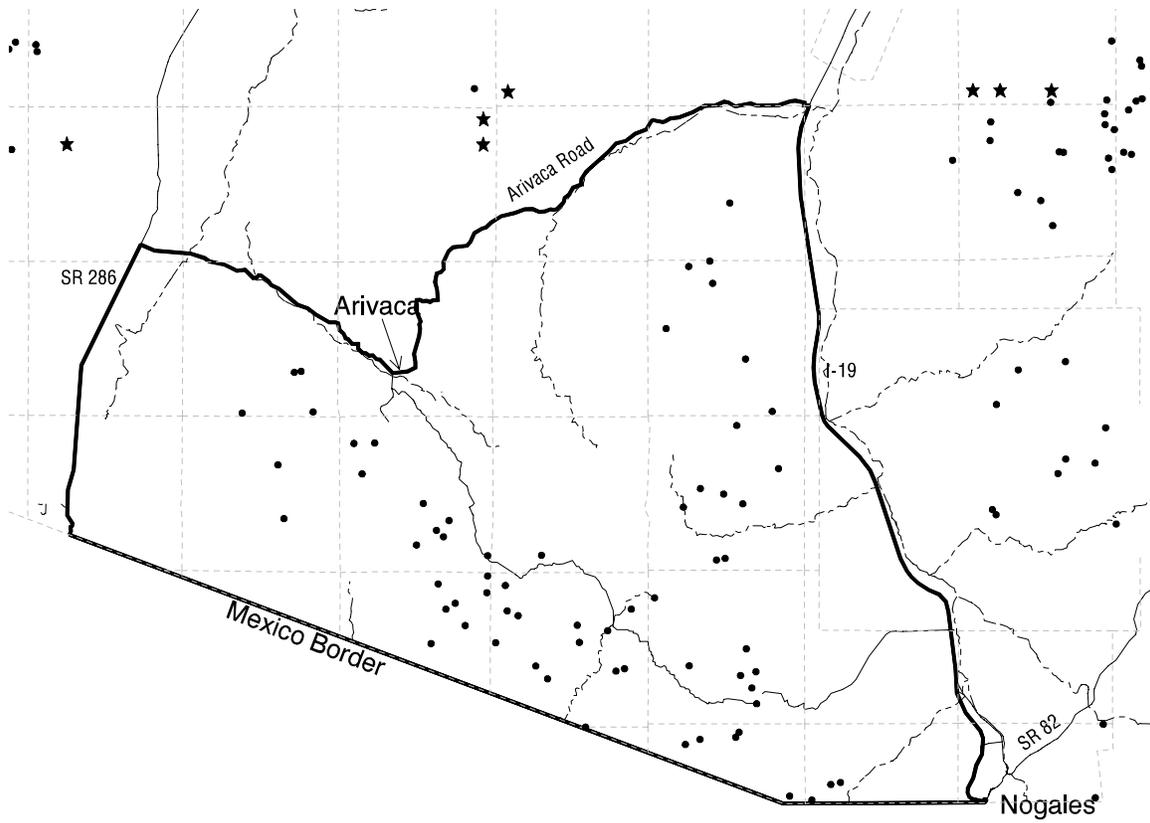


Figure 2 b. Water sources in GMU 1



**Legend**

- ⚡ Township/Range
- ▮ Unit Boundary
- ⚡ Roads
- ⚡ Streams
- Springs
- ★ AGFD Water Developments

**Game Management Unit 36B**

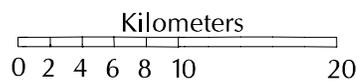
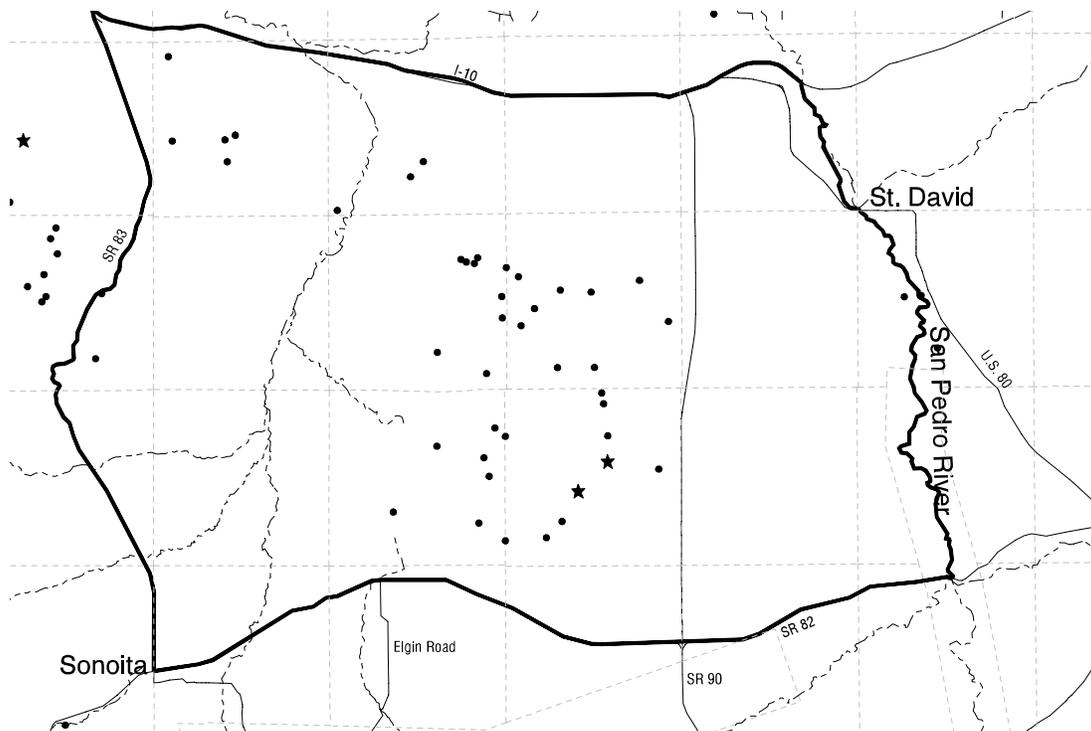


Figure 2 c. Water sources in GMU 36b



**Legend**

- Township/Range
- Unit Boundary
- Roads
- Streams
- Springs
- AGFD Water Developments

**Game Management Unit 34B**

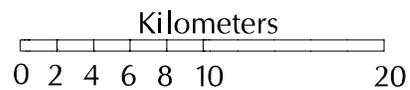


Figure 2 *d.* Water sources in GMU 34b.

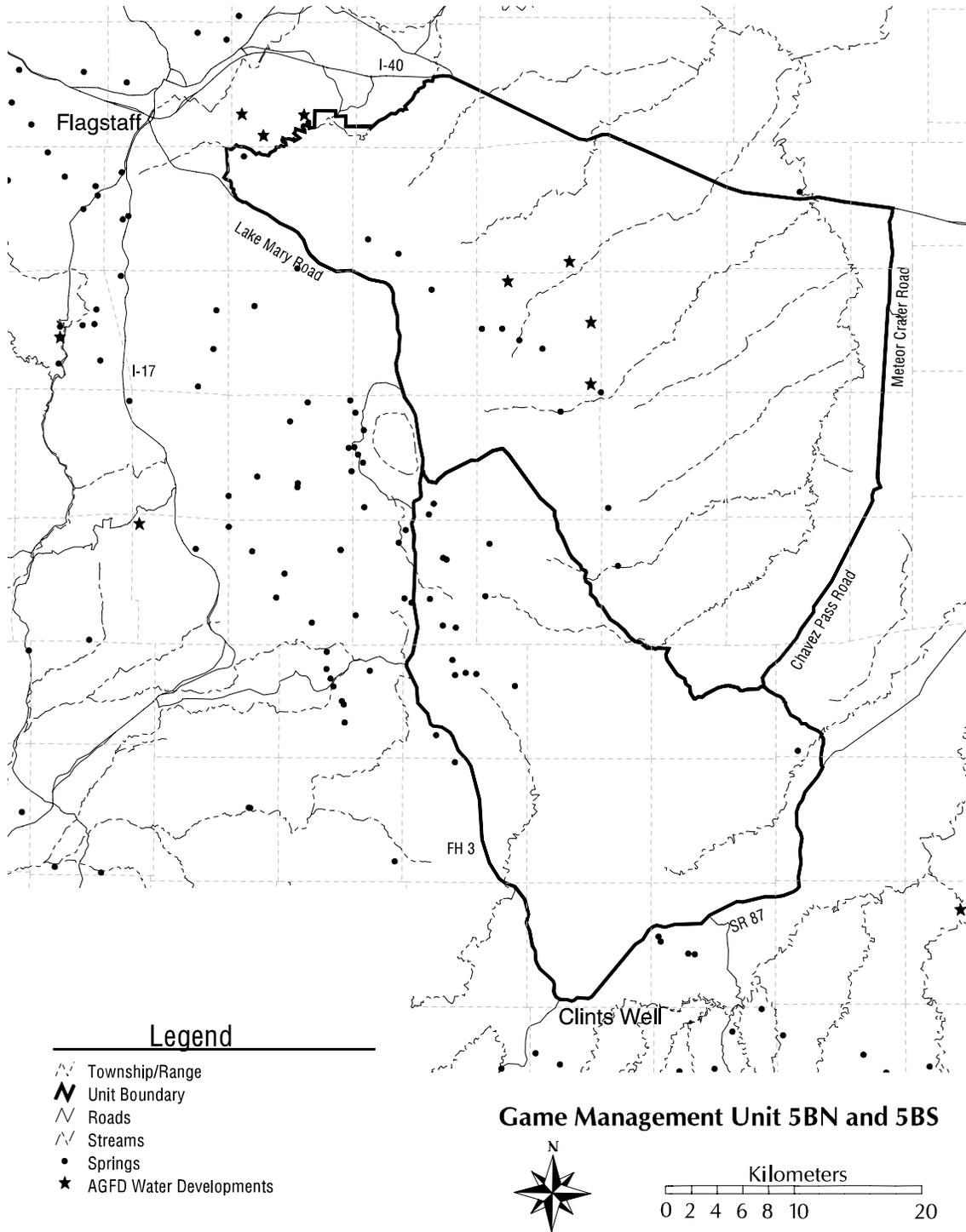
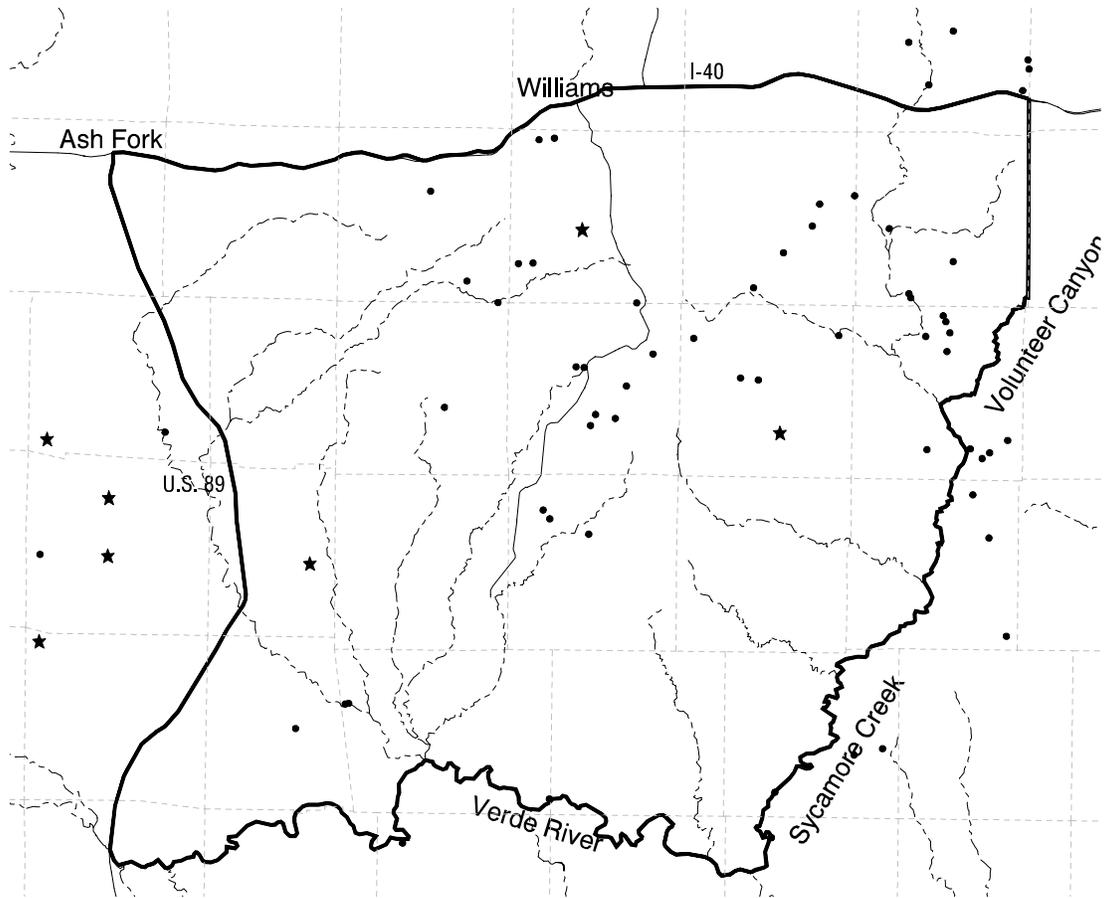


Figure 2 e. Water sources in GMU 5b



**Legend**

- Township/Range
- Unit Boundary
- Roads
- Streams
- Springs
- AGFD Water Developments

**Game Management Unit 8**

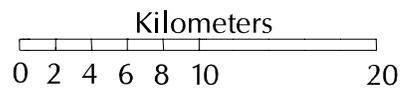
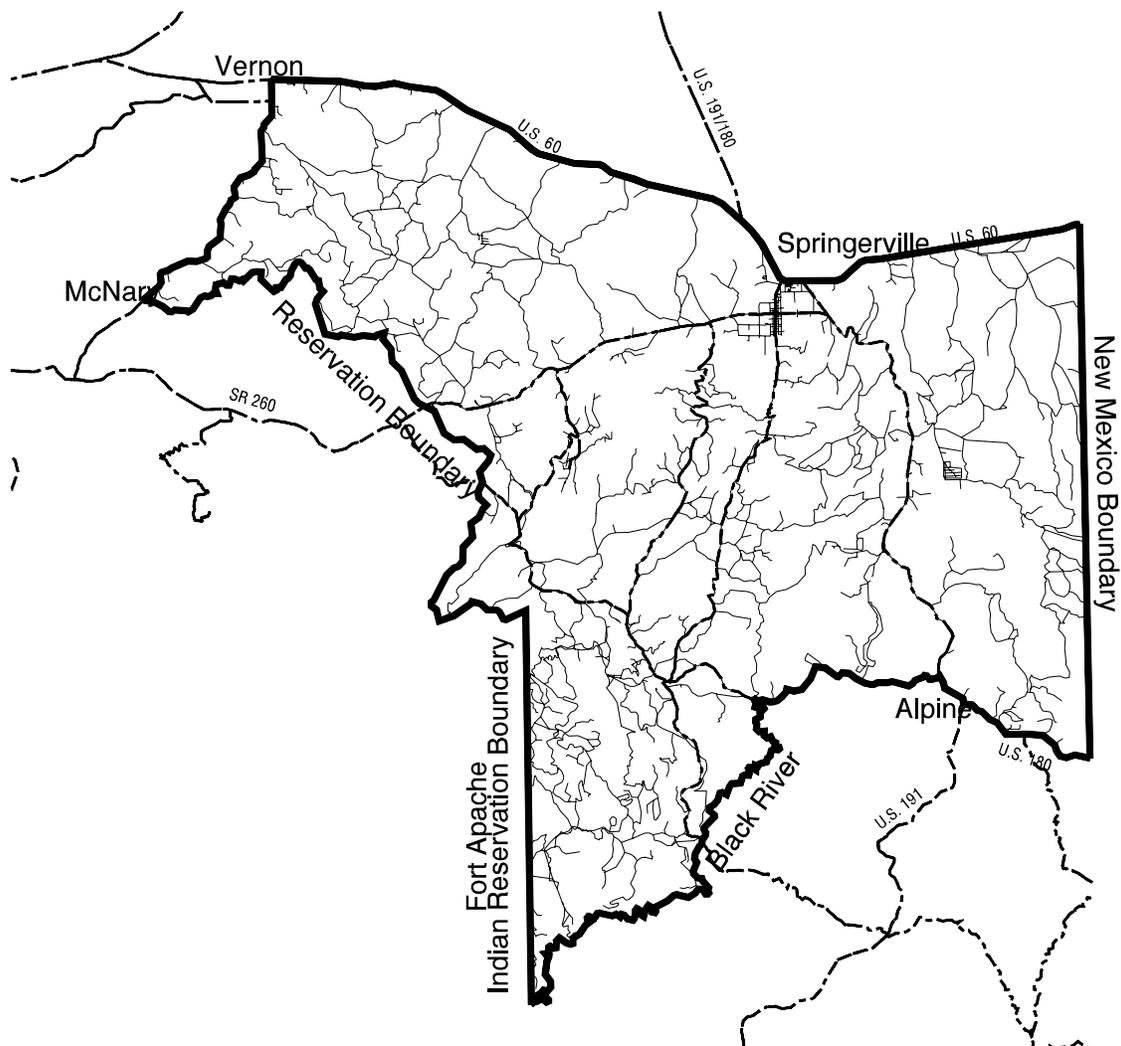


Figure 2 *f*. Water sources in GMU 8



**Legend**

-  Unit Boundary
-  Minor Trails and Roads
-  Main Roads

**Game Management Unit 1**

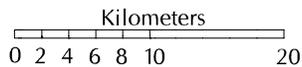


Figure 3 a. Roads in GMU 1

# Game Management Unit 2b

- Legend**
- Unit Boundary
  - Minor Trails and Roads
  - Main Roads

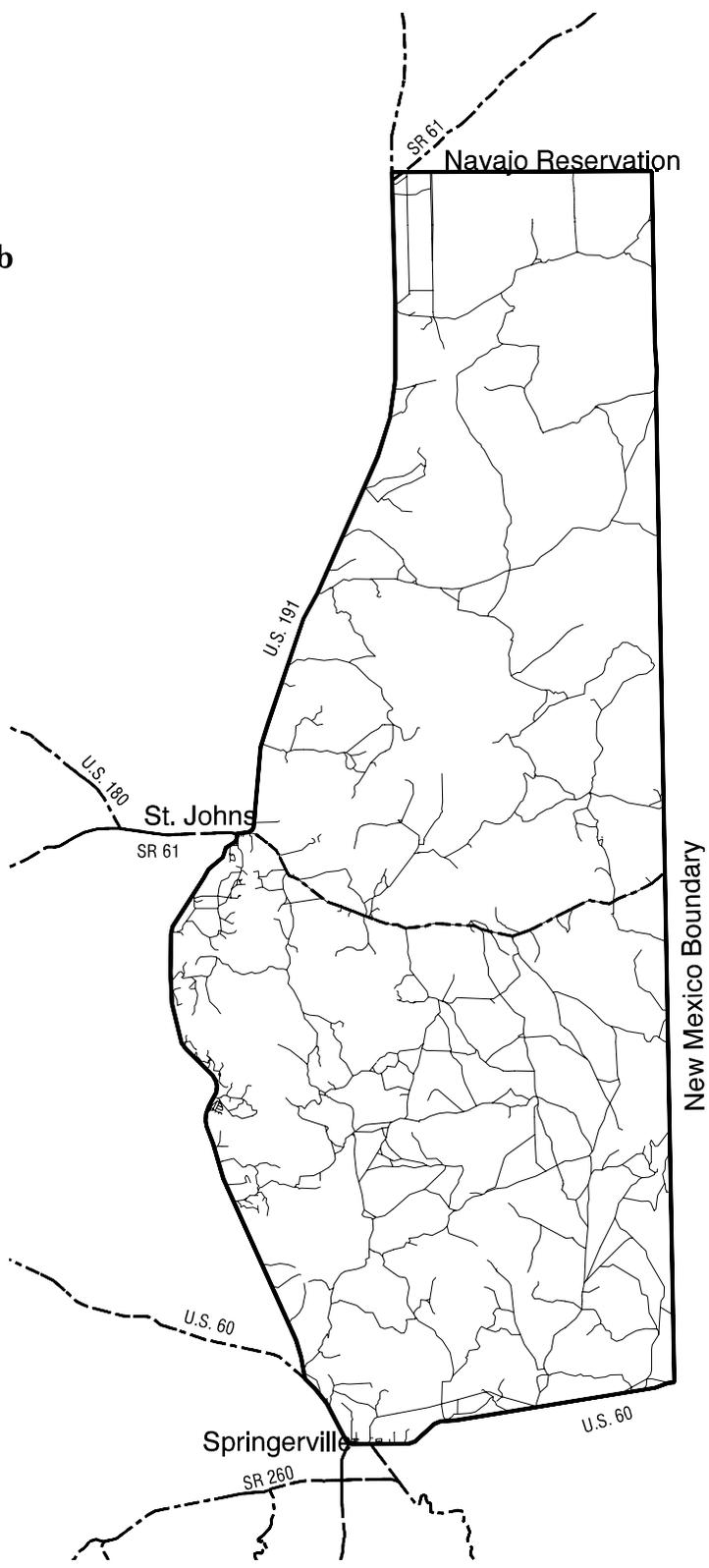
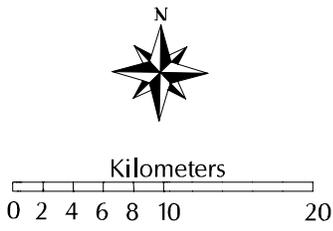
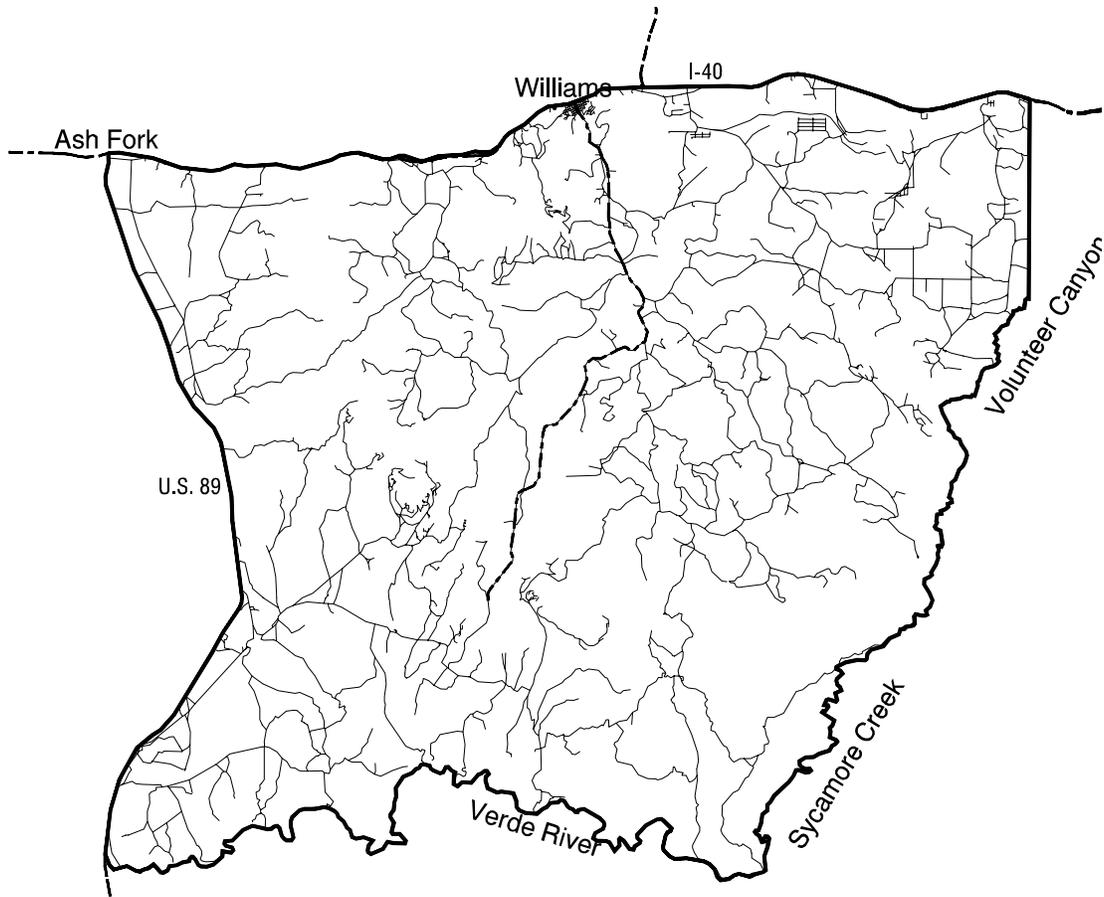


Figure 3 b. Roads in GMU 2b



**Legend**

-  Unit Boundary
-  Minor Trails and Roads
-  Main Roads

**Game Management Unit 8**



Kilometers

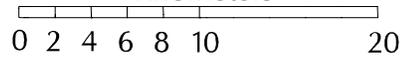


Figure 3 *c.* Roads in GMU 8

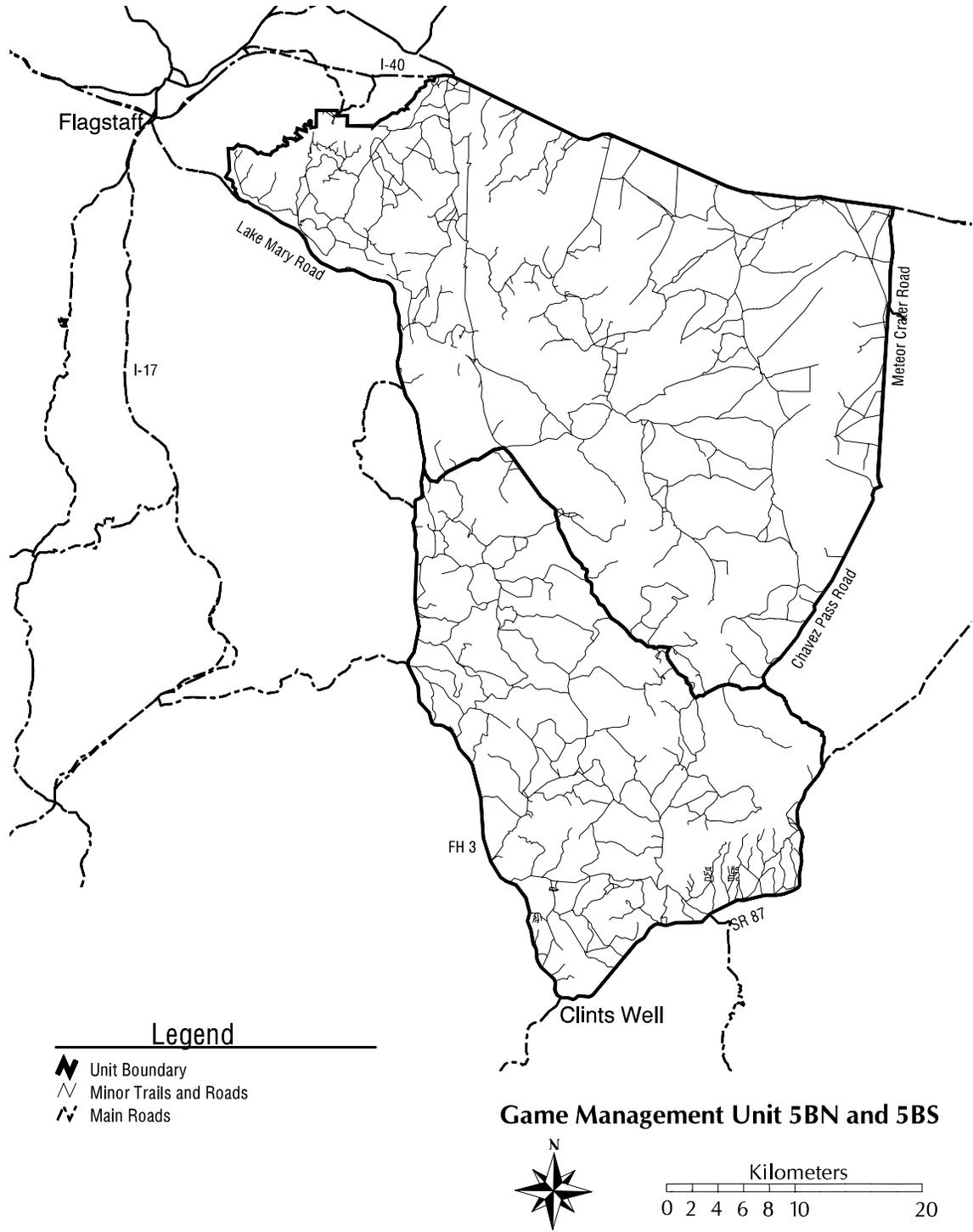
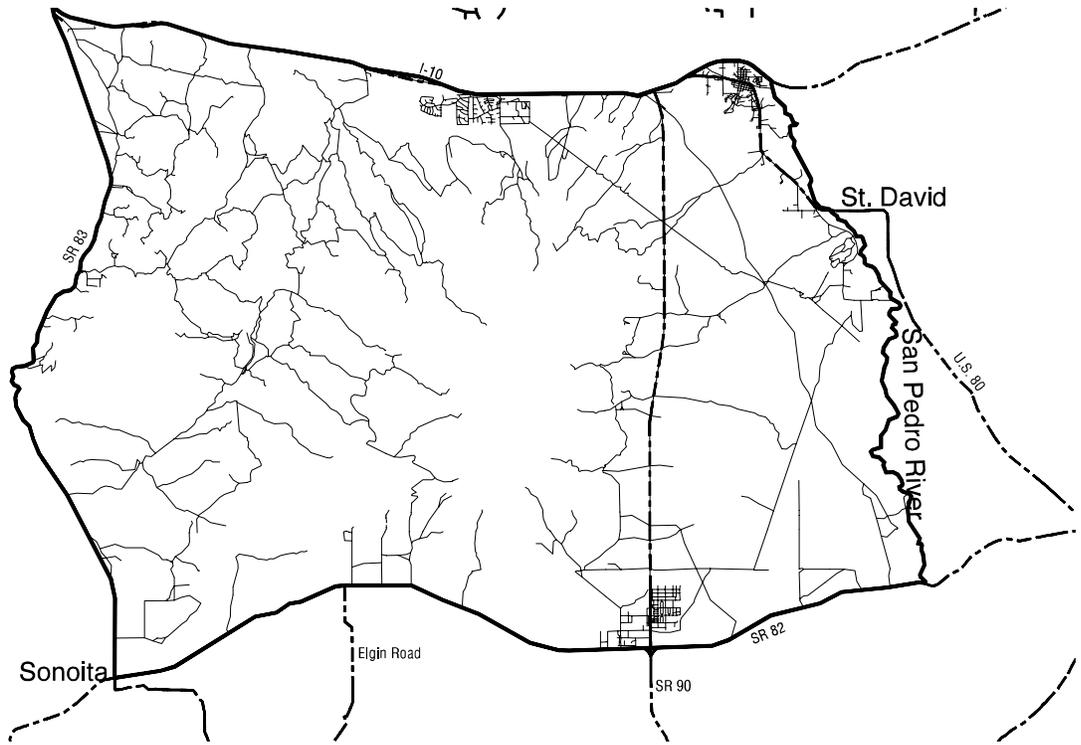


Figure 3 *d.* Roads in GMU 5b



**Legend**

-  Unit Boundary
-  Minor Trails and Roads
-  Main Roads

**Game Management Unit 34B**



Kilometers

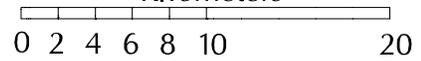
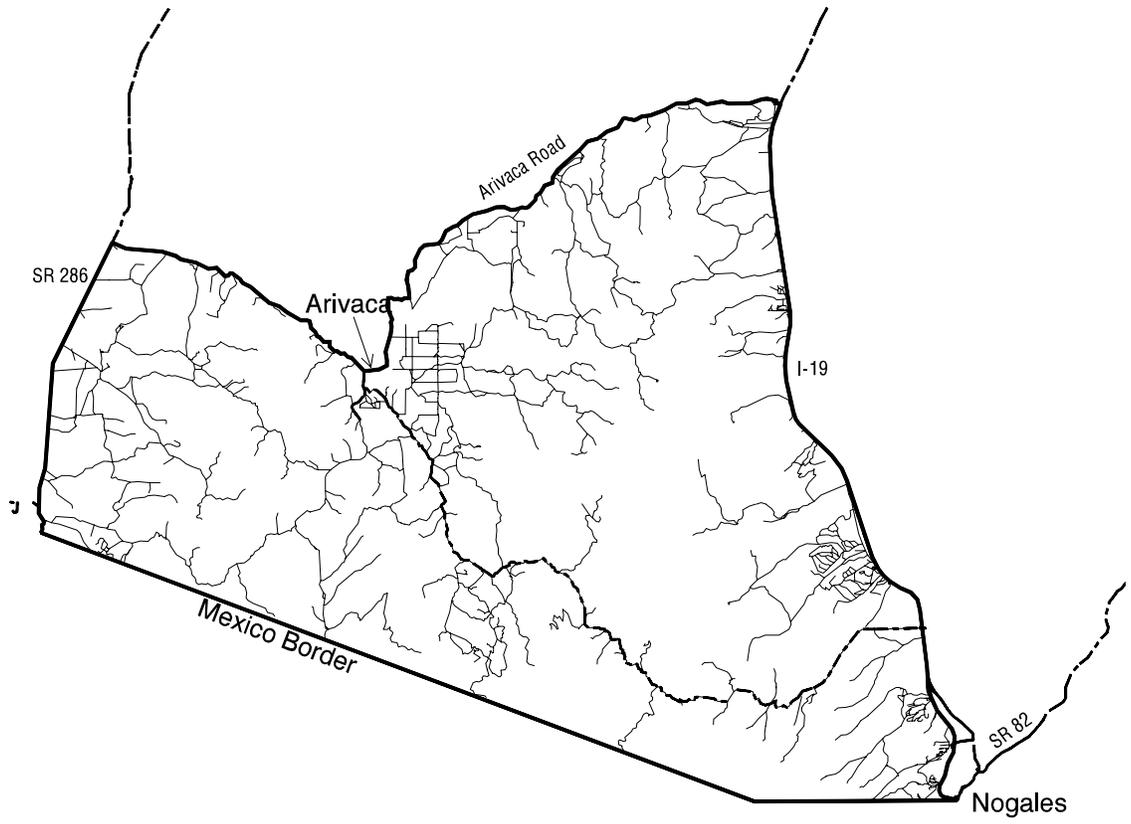


Figure 3 e. Roads in GMU 34b.



**Legend**

-  Unit Boundary
-  Minor Trails and Roads
-  Main Roads

**Game Management Unit 36B**

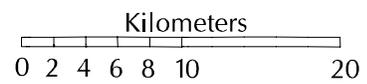


Figure 3 f. Roads in GMU 36b

Table 1. Schedule of tasks by month for 2001-2002.

Tasks	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Review literature	X	X	X	X	X	X	X	X				
Select/visit field sites		X	X	X	X		X	X				
Write study plan			X	X	X	X						
Develop steering committee						X	X					
Revise study plan							X	X				
Recreation										X	X	X
Disease surveillance			X	X								
Predator indices									X	X	X	
Water quality/availability									X	X	X	X
Fecal/plant collection									X	X	X	X
Nutritional analyses												X
Fawn hiding cover										X	X	
Acquire field equipment							X	X				
Data summary								X				

Table 2. Schedule of tasks by month for 2002-2003.

Tasks	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Data analysis and summary	X	X										
Annual report		X										
Fence structure				X	X							
Tree and shrub structure			X	X	X							
Disease surveillance			X	X								
Recreation	X	X	X							X	X	X
Predator indices									X	X	X	
Water quality/availability	X	X								X	X	X
Fecal/plant collection	X	X							X	X	X	X
Nutritional analyses	X	X	X	X	X	X	X	X				X
Fawn hiding cover										X	X	
Steering committee meeting					X							

Table 3. Schedule of tasks by month for 2003-2004.

Tasks	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Data analysis and summary	X	X										
Annual report		X										
Fence structure				X	X							
Tree and shrub structure			X	X	X							
Disease surveillance			X	X								
Recreation	X	X	X							X	X	X
Predator indices									X	X	X	
Water quality/availability	X	X								X	X	X
Fecal/plant collection	X	X							X	X	X	X
Nutritional analyses	X	X	X	X	X	X	X	X				X
Fawn hiding cover										X	X	
Steering committee meeting					X							

Table 4. Schedule of tasks by month for 2004-2005.

Tasks	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Literature review	X	X	X	X	X	X	X	X	X	X	X	X
Data summary and analysis	X	X										
Annual report		X										
Tree and shrub structure			X	X	X							
Disease surveillance			X	X								
Water quality/availability	X	X										
Fecal/plant collection	X	X										
Nutritional analyses	X	X	X	X	X							
Steering committee meeting					X							
Final report preparation						X	X	X	X	X	X	X
Print final report												X

Table 5. Technician time estimation for fawn hiding cover component in April and May.

Technician	Sites	Time	% total time for period
Kirby Bristow	30A	2 weeks	50
	36B	2 weeks	
Cindy Ticer	5B	2 weeks	50
	8	2 weeks	
Tim Rogers	1	2 weeks	50
	2B	2 weeks	

Table 6. Technician time estimation for predator indices component in April and May.

Technician	Sites	Time	% total time for period
Kirby Bristow	30A	2 weeks	50
	36B	2 weeks	
Stan Cunningham	5B	2 weeks	50
	8	2 weeks	
Lindsey Monroe	1	2 weeks	50
	2B	2 weeks	

Table 7. Technician time estimation for nutrition component in April and May.

Technician	Sites	Time	% total time for period
Tim Rogers	30A	2 weeks	50
	36B	2 weeks	
Cindy Ticer	5B	2 weeks	50
	8	2 weeks	
Shelli Dubay	1	2 weeks	50
	2B	2 weeks	

Table 8. Technician time estimation for water availability/quality component in June and July.

Technician	Sites	Time	% total time for period
Kirby Bristow	30A	2 weeks	50
	36B	2 weeks	
Tim Rogers	5B	2 weeks	50
	8	2 weeks	
Tim Rogers	1	2 weeks	50
	2B	2 weeks	

Table 9. Technician time estimation for nutrition component in June and July.

Technician	Sites	Time	% total time for period
Kirby Bristow	30A	2 weeks	50
	36B	2 weeks	
Cindy Ticer	5B	2 weeks	50
	8	2 weeks	
Shelli Dubay	1	2 weeks	50
	2B	2 weeks	

Table 10. Technician time estimation for water availability/quality component in August.

Technician	Sites	Time	% total time for period
Kirby Bristow	30A	1 week	50
	36B	1 week	
Tim Rogers	5B	1 week	50
	8	1 week	
Tim Rogers	1	1 week	50
	2B	1 week	

Table 11. Technician time estimation for nutrition component in August.

Technician	Sites	Time	% total time for period
Kirby Bristow	30A	1 week	50
	36B	1 week	
Cindy Ticer	5B	1 week	50
	8	1 week	
Shelli Dubay	1	1 week	50
	2B	1 week	

NOTE: Recreation component will be conducted concurrently with water component. Fences and tree and shrub cover will be conducted at off-peak periods so they were not included in the above technician time analysis.



September 10, 2001

Dear Pronghorn Antelope Hunter:

Congratulations on drawing a Region II-Flagstaff pronghorn antelope tag. This year, the Arizona Game & Fish Department is conducting a pronghorn health assessment at several sites. Your help in this study will aid wildlife management efforts. **Please use this vial to collect blood and use the whirl-pak bags to collect liver and hair samples from harvested antelope.** Please return the samples to us in one of the following ways:

- 1.) Give to a Wildlife Manager in the field after your hunt.
- 2.) Drop off at a check station after your hunt (see enclosed directions).
- 3.) Drop off at the Flagstaff Game & Fish office (3500 S. Lake Mary Road.). If dropping samples off during the weekend or after hours, please put samples in the cooler outside the door of the office.
- 4.) Call (928) 213-0961 for pickup in the Flagstaff area beginning 9-24.

**BLOOD:** Please fill the tube completely with the freshest and cleanest blood possible. Clean blood from the heart or chest cavity is best. Keep blood cool (place in refrigerator or ice chest as soon as possible), do not freeze or shake, and get it to us as soon as you can. The blood must be analyzed within a few days.

**LIVER:** Please place a piece of liver (about twice the size of your thumb) in the larger whirl-pak bag. Tear off the top perforated portion of the bag and place liver inside. Fold over the top of the bag using the yellow tabs and wrap the yellow tabs around the bag to close the bag. The liver is the large, lobed, dark red organ against the stomach (not the pinkish lungs in the chest cavity). The liver sample can be frozen but not the blood sample.

**HAIR:** Please place several hairs from between the shoulders in the other whirl-pak. It is important that the entire hair, from base to tip, is taken, so pluck the hair from just above the skin.

**LOCATION OF KILL:** Please record the location where you harvested your antelope to the best of your ability. A map of Unit 8 is located on the back of the letter. Please mark the kill location on this map and turn in with your samples.

We have only a few antelope harvested in this area so each sample is *extremely* important. Thank you for your help in this important effort and good luck.

Sincerely,

Shelli Dubay  
Research Biologist  
(602) 789-3351

Enclosure

cc: John Goodwin, Rick Miller, Carl Lutch, Dan Gaska