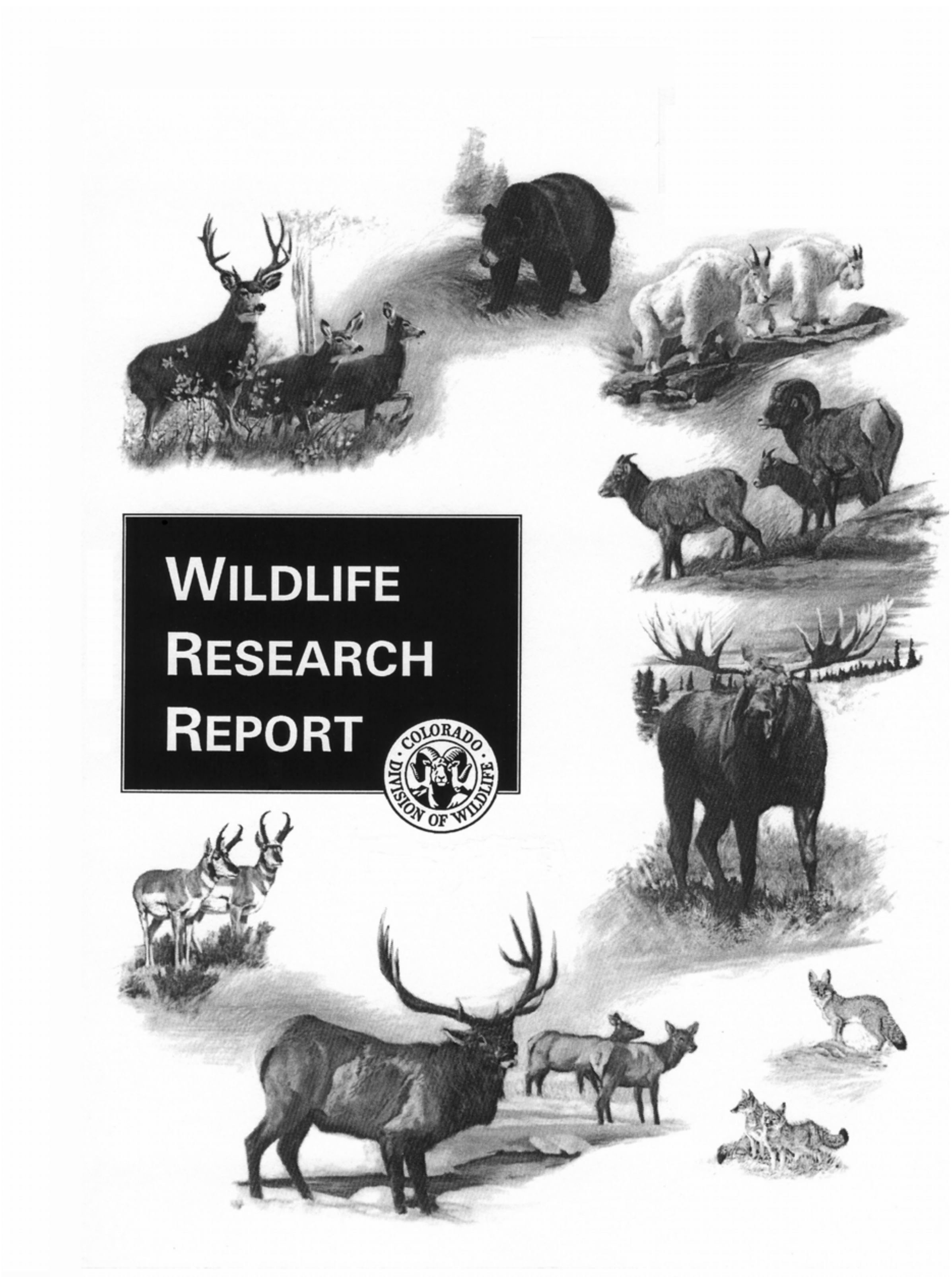
MAMMALS - JULY 2005



WILDLIFE RESEARCH REPORTS

JULY 2004 – JUNE 2005

MAMMALS PROGRAM

COLORADO DIVISION OF WILDLIFE
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Colorado Division of Wildlife
July 2004 – June 2005

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Cost Center	3430	:	Mammals Research
Work Package	0670	:	Lynx Conservation
Task No.	1	:	Post-Release Monitoring of Lynx Reintroduced to Colorado
Federal Aid Project:	N/A	:	

Period Covered: July 1, 2004- June 30, 2005

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ABSTRACT

In an effort to establish a viable population of lynx (*Lynx canadensis*) in Colorado, the Colorado Division of Wildlife (CDOW) initiated a reintroduction effort in 1997 with the first lynx released in February 1999. A total of 166 lynx were released from 1999-2004 and an augmentation of 38 additional animals (20 males:18 females) was completed in 2005 resulting in a total of 204 lynx reintroduced to southwestern Colorado. Each lynx was released with dual satellite and VHF radio transmitters to allow intensive monitoring of animals after release. Locations of each lynx were collected through aerial- or satellite-tracking to document movement patterns. Most lynx remain in the southwestern quarter of Colorado. Through documentation of lynx mortalities and causes of death, human-caused mortality factors, such as gunshot and vehicle collision, are currently the highest source of mortality for reintroduced lynx. Reproduction was first documented during the 2003 reproduction season with 6 dens and 16 kittens found. A second successful breeding season was documented in 2004 with 30 kittens found at 11 dens and an addition 9 kittens found after denning season. In 2005, 46 kittens were found at 16 dens with an additional den located but not visited for safety reasons. Data collected from snow-tracking indicate the primary winter prey species are snowshoe hare (*Lepus americanus*) and red squirrel (*Tamiasciurus hudsonicus*), with other mammals and birds forming a minor part of the winter diet. Site-scale habitat data collected from snow-tracking efforts indicate Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) are the most common forest stands used by lynx in southwestern Colorado. Results to date have demonstrated that CDOW has developed release protocols that ensure high initial post-release survival, and on an individual level, lynx have demonstrated an ability to survive long-term in areas of Colorado. Reintroduced lynx have also exhibited site fidelity, engaged in breeding behavior and produced kittens. What is yet to be demonstrated is whether conditions in Colorado can support the recruitment necessary to offset annual mortality for a population to remain viable for several generations of lynx. Monitoring of reintroduced lynx will continue in an effort to document such viability.

WILDLIFE RESEARCH REPORT

POST RELEASE MONITORING OF LYNX (*LYNX CANADENSIS*) REINTRODUCED TO COLORADO

TANYA M. SHENK

P. N. OBJECTIVE

The initial post-release monitoring of lynx reintroduced into Colorado will emphasize 5 primary objectives:

1. Assess and modify release protocols to ensure the highest probability of survival for each lynx released.
2. Obtain regular locations of released lynx to describe general movement patterns and habitats used by lynx.
3. Determine causes of mortality in reintroduced lynx.
4. Estimate survival of lynx reintroduced to Colorado.
5. Estimate reproduction of lynx reintroduced to Colorado.

Three additional objectives will be emphasized after lynx display site fidelity to an area:

6. Refine descriptions of habitats used by reintroduced lynx.
7. Refine descriptions of daily and overall movement patterns of reintroduced lynx.
8. Describe hunting habits and prey of reintroduced lynx.

Information gained to achieve these objectives will form a basis for the development of lynx conservation strategies in the southern Rocky Mountains.

SEGMENT OBJECTIVES

1. Release additional adult lynx captured in Canada in southwestern Colorado during spring 2005.
2. Complete winter 2004-05 field data collection on lynx habitat use, hunting behavior, diet, mortalities, and movement patterns.
3. Complete winter 2004-05 lynx trapping field season to collar Colorado born lynx and re-collar adult lynx.
4. Complete spring 2005 field data on lynx reproduction.
5. Summarize and analyze data and publish information as Progress Reports, peer-reviewed manuscripts for appropriate scientific journals, or CDOW technical publications.

INTRODUCTION

The Canada lynx occurs throughout the boreal forests of northern North America. Colorado represents the southern-most historical distribution of lynx, where the species occupied the higher elevation, montane forests in the state. Little was known about the population dynamics or habitat use of this species in their southern distribution. Lynx were extirpated or reduced to a few animals in the state by the late 1970's due, most likely, to predator control efforts such as poisoning and trapping. Given the isolation of Colorado to the nearest northern populations, the CDOW considered reintroduction as the only option to attempt to reestablish the species in the state.

A reintroduction effort was begun in 1997, with the first lynx released in Colorado in 1999. To date, 204 wild-caught lynx from Alaska and Canada have been released in southwestern Colorado. The goal of the Colorado lynx reintroduction program is to establish a self-sustaining, viable population of lynx in this state. Evaluation of incremental achievements necessary for establishing viable populations is an interim method of assessing if the reintroduction effort is progressing towards success. There are 7 critical criteria for achieving a viable population: 1) development of release protocols that lead to a high initial post-release survival of reintroduced animals, 2) long-term survival of lynx in Colorado, 3) development of site fidelity by the lynx to areas supporting good habitat in densities sufficient to breed, 4) reintroduced lynx must breed, 5) breeding must lead to reproduction of surviving kittens 6) lynx born in Colorado must reach breeding age and reproduce successfully, and 7) recruitment must be equal to or greater than mortality.

The post-release monitoring program for the reintroduced lynx has 2 primary goals. The first goal is to determine how many lynx remain in Colorado and their locations relative to each other. Given this information and knowing the sex of each individual, we can assess whether these lynx can form a breeding core from which a viable population might be established. From these data we can also describe general movement patterns and habitat use. The second primary goal of the monitoring program is to estimate survival of the reintroduced lynx and, where possible, determine causes of mortality for reintroduced lynx. Such information will help in assessing and modifying release protocols and management of lynx once they have been released to ensure their highest probability of survival.

Additional goals of the post-release monitoring program for lynx reintroduced to the southern Rocky Mountains included refining descriptions of habitat use and movement patterns and describing successful hunting habitat once lynx established home ranges that encompassed their preferred habitat. Specific objectives for the site-scale habitat data collection include: 1) describe and quantify site-scale habitat use by lynx reintroduced to Colorado, 2) compare site-scale habitat use among types of sites (e.g., kills vs. long-duration beds), and 3) compare habitat features at successful and unsuccessful snowshoe hare chases. The program will also investigate the ecology of snowshoe hare in Colorado.

Documenting reproduction is critical to the success of the program and lynx are monitored intensively to document breeding, births, survival and recruitment of lynx born in Colorado. Site-scale habitat descriptions of den sites are also collected and compared to other sites used by lynx.

Lynx is listed as threatened under the Endangered Species Act (ESA) of 1973, as amended (16 U. S. C. 1531 et. seq.)(U. S. Fish and Wildlife Service 2000). Colorado is included in the federal listing as lynx habitat. Thus, an additional objective of the post-release monitoring program is to develop conservation strategies relevant to lynx in Colorado. To develop these conservation strategies, information specific to the ecology of the lynx in its southern Rocky Mountain range, such as habitat use, movement patterns, mortality factors, survival, and reproduction in Colorado is needed.

STUDY AREA

Southwestern Colorado is characterized by wide plateaus, river valleys, and rugged mountains that reach elevations over 4200 m. Engelmann spruce-subalpine fir is the most widely distributed coniferous forest type at elevations most typically used by lynx. The Core Research Area is defined as areas bounded by the New Mexico state line to the south, Taylor Mesa to the west and Monarch Pass on the north and east and > 2900 meters in elevation.

METHODS

REINTRODUCTION

Effort

All 2005 lynx releases were conducted under the protocols found to maximize survival (see Shenk 2001). Estimated age, sex and body condition were ascertained and recorded for each lynx prior to release (see Wild 1999). Specific release sites were those used in earlier years of the project and were selected based on land ownership and accessibility during times of release (Byrne 1998). Lynx were transported from the Frisco Creek Wildlife Rehabilitation Center, where they were held from their time of arrival in Colorado, to their release site in individual cages. Release site location was recorded in Universal Transverse Mercator (UTM) coordinates and identification of all lynx released at the same location, on the same day, was recorded. Behavior of the lynx on release and movement away from the release site were documented.

Distribution and Movement Patterns

All lynx released in 1999 were fitted with Telonics™ radio-collars. All lynx released since 1999, with the exception of 5 males released in spring 2000, were fitted with Sirtrack™ dual satellite/VHF radio-collars. These collars have a mortality indicator switch that operated on both the satellite and VHF mode. The satellite component of each collar was programmed to be active for 12 hours per week. The 12-hour active periods for individual collars were staggered throughout the week. Signals from the collars allowed for locations of the animals to be made via Argos, NASA, and NOAA satellites. The location information was processed by ServiceArgos and distributed to the CDOW through e-mail messages.

To determine general movement patterns of reintroduced lynx, regular locations of released lynx were collected through a combination of aerial, satellite and ground radio-tracking. Locations were recorded in UTM coordinates and general habitat descriptions for each ground and aerial location were recorded.

Survival and Mortality Factors

When a mortality signal (75 beats per minute [bpm] vs. 50 bpm for the Telonics™ VHF transmitters, 20 bpm vs. 40 bpm for the Sirtrack™ VHF transmitters, 0 activity for Sirtrack™ PTT) was heard during either satellite, aerial or ground surveys, the location (UTM coordinates) was recorded. Ground crews then located and retrieved the carcass as soon as possible. The immediate area was searched for evidence of other predators and the carcass photographed in place before removal. Additionally, the mortality site was described and habitat associations and exact location were recorded. Any scat found near the dead lynx that appeared to be from the lynx was collected.

All carcasses were transported to the Colorado State University Veterinary Teaching Hospital (CSUVTH) for a post mortem exam to 1) determine the cause of death and document with evidence, 2) collect samples for a variety of research projects, and 3) archive samples for future reference (research or forensic). The gross necropsy and histology were performed by, or under the lead and direct supervision of a board certified veterinary pathologist. At least one research personnel from the CDOW involved with the lynx program was also present. The protocol followed standard procedures used for thorough post-mortem examination and sample collection for histopathology and diagnostic testing (see Shenk 1999 for details). Some additional data/samples were routinely collected for research, forensics, and archiving. Other data/samples were collected based on the circumstances of the death (e.g., photographs, video, radiographs, bullet recovery, samples for toxicology or other diagnostic tests, etc.).

From 1999–2004 the CDOW retained all samples and carcass remains with the exception of tissues in formalin for histopathology, brain for rabies exam, feces for parasitology, external parasites for ID, and other diagnostic samples. Since 2005 carcasses are disposed of at the CSUVTH with the exception of the lower canine, fecal samples, stomach content samples and tissue or bone marrow samples to be delivered by CDOW to the Center for Disease control for plague testing. The lower canine is sent to Matson Labs (Missoula, Montana) for aging and the fecal and stomach content samples are evaluated for diet.

Reproduction

Females were monitored for proximity to males during each breeding season. We defined a possible mating pair as any male and female documented within at least 1 km of each other in breeding season through either flight data or snow-tracking data. Females were then monitored for site fidelity to a given area during each denning period of May and June. Each female that exhibited stationary movement patterns in May or June were closely monitored to locate possible dens. Dens were found when field crews walked in on females that exhibited virtually no movement for at least 10 days from both aerial and ground telemetry.

Kittens found at den sites were weighed, sexed and photographed. Each kitten was uniquely marked by inserting a sterile passive integrated transponder (PIT, Biomark, Inc., Boise, Idaho, USA) tag subcutaneously between the shoulder blades. Time spent at the den was minimized to ensure the least amount of disturbance to the female and the kittens. Weight, PIT-tag number, sex and any distinguishing characteristics of each kitten was also recorded. Beginning in 2005, blood and saliva samples were collected for genetic identification.

During the den site visits, den site location was recorded as UTM coordinates. General vegetation characteristics, elevation, weather, field personnel, time at the den, and behavioral responses of the kittens and female were also recorded. Once the females moved the kittens from the natal den area, den sites were visited again and site-specific habitat data were collected (see Habitat Use section below).

Recaptures

Recaptures were attempted for either lynx that were in poor body condition or lynx that needed to have their radio-collars replaced due to failed or failing batteries or to radio-collar kittens born in Colorado once they reached at least 10-months of age when they were nearly adult size. Methods of recapture included 1) trapping using a Tomahawk™ live trap baited with a rabbit and visual and scent lures, 2) calling in and darting lynx using a Dan-Inject CO_2 rifle, 3) custom box-traps modified from those designed by other lynx researchers (Kolbe et al. 2003) and 4) hounds trained to pursue felids were also used to tree lynx and then the lynx was darted while treed. Lynx were immobilized either with Telazol (3 mg/kg; modified from Poole et al. 1993 as recommended by M. Wild, DVM) or medetomidine (0.09mg/kg) and ketamine (3 mg/kg; as recommended by L. Wolfe, DVM)) administered intramuscularly (IM) with either an extendible pole-syringe or a pressurized syringe-dart fired from a Dan-Inject air rifle.

Immobilized lynx were monitored continuously for decreased respiration or hypothermia. If a lynx exhibited decreased respiration 2mg/kg of Dopram was administered under the tongue; if respiration was severely decreased, the animal was ventilated with a resuscitation bag. If medetomidine/ketamine were the immobilization drugs, the antagonist Atipamezole hydrochloride (Antisedan) was administered. Hypothermic (body temperature < 95° F) animals were warmed with hand warmers and blankets.

While immobilized, lynx were fitted with replacement Sirtrack™ VHF/satellite collar and blood and hair samples were collected. Once an animal was processed, recovery was expedited by injecting the equivalent amount of the antagonist Antisedan IM as the amount of medetomidine given, if medetomodine/ketemine was used for immobilization. Lynx were then monitored while confined in the box-trap until they were sufficiently recovered to move safely on their own. No antagonist is available for Telezol so lynx anesthetized with this drug were monitored until the animal recovered on its own in the box-trap and then released. If captured and in poor body condition lynx were anesthetized with Telezol (2 mg/kg) and returned to the Frisco Creek Wildlife Rehabilitation Center for treatment.

HABITAT USE

Gross habitat use was documented by recording canopy vegetation at aerial locations. More refined descriptions of habitat use by reintroduced lynx were obtained through following lynx tracks in the snow (i.e., snow-tracking) and site-scale habitat data collection conducted at sites found through this method to be used by lynx.

Snow-tracking

Locations from aerial- and satellite-tracking were used to help ground-trackers locate lynx tracks in snow. Snowmobiles, where permitted, were used to gain the closest possible access to the lynx tracks without disturbing the animal. From that point, the tracking team used snowshoes to access tracks. Once tracks were found, the ground crew back- or forward-tracked the animal if it was far enough away not to be disturbed. Back-tracking generally avoided the possibility of disturbing the lynx by moving away from the animal rather than towards the animal. However, monitoring of the lynx through radio-telemetry was used to assure that the ground crew was staying a sufficient distance away from the lynx in the event the lynx might double back on its tracks. Radio-telemetry was also used in forward-tracking to make sure the team did not disturb the animal. If it appeared the lynx began to move in response to the observers, the observers stopped following the tracks. If the lynx began to move and the movement did not appear to be a response to the observers, the ground crew continued following the track.

An attempt was made in Season 1 (February-May 1999) and Season 2 (December 1999-April 2000) to snow-track each lynx. In Season 3 (December 2000-April 2001), we attempted to snow-track all lynx within the Core Research Area. In tracking Season 4 (December 2001-April 2002), Season 5 (December 2002-April 2003), Season 6 (December 2003-April 2004) and Season 7 (December 2004-April 2005) we attempted to track all accessible lynx in the Core Research Area and some lynx north of the Core Research Area. Ground crews were instructed to track lynx only where it was safe to travel. Restrictions to safe travel included avalanche danger and extremely rugged terrain. Ground crews worked in pairs and were fully equipped for winter back-country survival.

Data Collection

For each day of tracking the date, lynx being tracked, slope, aspect, UTM coordinates, elevation, general habitat description, and summary of the days tracking were recorded. Aspect was defined as the direction of 'downhill' or 'fall line' on a slope. This is the direction along the ground in a dihedral angle between the horizontal and the plane of the ground surface. Units were compass degrees. Slope was defined as the dihedral angle between the horizontal and the plane of the ground surface (e.g., 45").

Once a track was located there were 2 types of 'sites' that were encountered. Site I areas needed documentation but either did not reflect areas lynx selected for specific habitat features, or were sites that occurred too frequently to measure each in detail. These sites included the start and end of the track being followed, the location of scat, and short-duration beds defined as being small in size (approximating an area a lynx would crouch), and with little ice formed in the bed indicating little time spent there. Site II areas included areas that might reflect specific habitat features lynx selected for and included locations where the following were found: kills, start of chases, territory marks (e.g., spray sites, buried scat, scat placed on prominent locations), long-duration beds (encompasses an area where a lynx would have lain for an extended period, iced bottom), and road crossing (both sides of road). In addition, habitat plots were conducted along lynx travel routes if no other sites sampled in last hour.

At each of the 2 types of sites the date, lynx tracked, slope, aspect, forest structure class, UTM coordinates, and elevation were recorded. Forest structure classes included grass/forb, shrub/seedling, sapling/pole, mature, and old growth as defined in Table 1. For Site I areas, the only additional data that was collected was identification of what the site was used for (e.g., short-duration bed), and a brief description of the site. Habitat plots (see below) were conducted at Site II areas.

Description of the Habitat Plot

The habitat plot consisted of a 12 m x 12 m square defined by a series of 25 points placed in 5 rows of 5 with the center point being on the object that defined the site (e.g., a kill)(Figure 1). Each point was 3 m apart. The 12 m x 12 m sampling square exceeded the minimum requirement of 0.01 ha. recommended by Curtis (1959) for sampling trees.

Measurements taken at each of the 25 points included:

1. Snow depth - measured vertically by an avalanche probe marked in cm.
2. Understory - measured from top of snow to 150 cm above snow in a column of 3-cm radius around the avalanche probe. Because understory measurements were influenced by vegetation outside the perimeter of the 25 sampling points (12 m x 12 m) the area used for estimating undersory cover was 15 m by 15 m. At each point, crews recorded all shrubs, trees and coarse woody debris (CWD) that fell within this column and was visible above the snow. Crews also recorded number of branches of each species that fell within the column at 3 different height categories (0-0.5 m, 0.51-1.0 m, 1.01-1.5 m).
3. Overstory: measured at 150 cm above snow with a sighting tube. The tube was made of PVC pipe, with a curved viewing end and a crosshair made of wire on the opposite end. The sighting tube was attached to the avalanche probe used to measure snow depth. Species that hit the crosshair were recorded at each of the 25 points in the vegetation plot. Ganey and Block (1994) found this method of measuring canopy cover (with 20 sample points per plot; Laymon 1988) provided greater precision among observers.
4. Species composition: all the different species of tree or shrub that hit the crosshair of the sighting tube at each of the 25 points were recorded.
5. Tree composition of the vegetation plot was recorded by species and diameter at breast height (DBH). Snow depth was used in conjunction with this recorded DBH to estimate true DBH. Within the 12 m x 12 m square all conifers and deciduous trees were recorded by DBH size class (A = 0-6 in, B = 6.1-12 in, C = 12.1 -18 in, D = 18.1-24 in, E = > 24 in). Area for the tree composition analysis was 12 m x 12 m.

Understory was estimated as: 1) percent occurrence within the vegetation plot (number of points with understory/total number of points surveyed) and 2) mean percent occurrence and variance by species and height category over the total points sampled within the vegetation plot.
Overstory was estimated as percent occurrence over the vegetation plot (number of points with overstory/total number of points surveyed).

DIET AND HUNTING BEHAVIOR

Winter diet of reintroduced lynx was estimated by documenting successful kills through snow-tracking. Prey species from failed and successful hunting attempts were identified by either tracks or remains. Scat analysis also provided information on foods consumed. Scat samples were collected wherever found and labeled with location and individual lynx identification. Only part of the scat was collected (approximately 75%); the remainder was left in place in the event that the scat was being used by the animal as a territory mark. Site-scale habitat data collected for successful and unsuccessful snowshoe hare kills were compared.

RESULTS

REINTRODUCTION

Effort

From 1999 through 2004 166 lynx were reintroduced into southwestern Colorado. An additional 37 lynx were released in April 2005 (17 females and 20 males), one female was released in June 2005. This brings the total number of lynx released in Colorado to 204 (Table 2). These lynx released in 2005 were captured in Quebec, British Columbia and Manitoba. All lynx were released in the Core Research

Area of southwestern Colorado at or near previously used release sites in southwestern Colorado. Lynx were released with dual VHF/satellite radio collars so they can be monitored for movement and mortality. The CDOW plans to release up to 15 lynx annually from 2006-2008.

Distribution and Movement Patterns

A total of 7421 aerial VHF locations for all 204 reintroduced lynx have been collected to date. An additional 14,788 satellite locations have been collected. Most lynx released remained in the southwestern quarter of Colorado. The majority of surviving lynx from the entire reintroduction effort continue to use areas from New Mexico north to Gunnison, west as far as Taylor Mesa and east to Monarch Pass. Most movements away from the Core Research Area were to the north.

Numerous travel corridors have been used repeatedly by more than one lynx. These travel corridors include the Cochetopa Hills area for northerly movements, the Rio Grande Reservoir-Silverton-Lizardhead Pass for movements to the west, and southerly movements down the east side of Wolf Creek Pass to the southeast through the Conejos River Valley. Lynx appear to remain faithful to an area during winter months, and exhibit more extensive movements away from these areas in the summer. Such movement patterns have also been documented by native lynx in Wyoming and Montana (Squires and Laurion 1999).

Survival and Mortality Factors

Of the total 204 adult lynx released from 1999-2005 there are 66 known mortalities. Of these 66 mortalities, 26 are from the 1999 releases, 24 are from the 2000 releases, 5 are from the 2003 releases, 8 are from the 2004 releases, and 3 are from the 2005 releases. Causes of death are listed in Table 3. Starvation was a significant cause of mortality in the first year of releases only. Mortalities occurred throughout the areas through which lynx moved.

As of June 30, 2005, CDOW was actively tracking 110 of the 138 lynx still possibly alive. There are 29 lynx that we have not heard signals on since at least June 30, 2004 and these animals are classified as 'missing' (Table 4). One of these missing lynx is a mortality of unknown identity, thus only 28 are truly missing. Possible reasons for not locating these missing lynx include 1) long distance dispersal, beyond the areas currently being searched, 2) radio failure, or 3) destruction of the radio (e.g., run over by car). CDOW continues to search for all missing lynx during both aerial and ground searches. Two of the missing lynx released in 2000 are thought to have slipped their collars.

Reproduction

2003.-- Nine pairs of lynx were documented during the 2003 breeding season (March and April). In May and June, 6 dens and a total of 16 kittens were found in the lynx Core Research Area in southwestern Colorado (Table 5). At all dens the females appeared in excellent condition, as did the kittens. The kittens weighed from 270-500 grams. Lynx kittens weigh approximately 200 grams at birth and do not open their eyes until they are 10-17 days old.

The dens were scattered throughout the Core Research Area, with no dens found outside the core area. All the dens were in Engelmann spruce/subalpine fir forests in areas of extensive downfall. Elevations ranged from 3240-3557 m. Field crews weighed, photographed, PIT-tagged the kittens and . took hair samples from the kittens for genetic work in an attempt to confirm paternity. Kittens were processed as quickly as possible (11-32 minutes) to minimize the time the kittens were without their mother. While working with the kittens the females remained nearby, often making themselves visible to the field crews. The females generally continued a low growling vocalization the entire time personnel were at the den. In all cases, the female returned to the den site once field crews left the area.

Four of the 6 females that we know had kittens in summer 2003 were still with kittens at the end of April 2004. Two of those females still had 2 kittens with them at that time. Visual observations in February 2004 of one female with 2 kittens indicated all 3 were in good body condition. The mortality of female YK00F16 and her 1 kitten in October 2003 from plague was not due to poor habitat or prey conditions, and thus we might assume she would have raised the 1 kitten to this stage as well. Three probable kitten deaths from female YK00F19 were from 1 litter that most likely failed very early. Through snow-tracking in winter 2003-04 an unknown female (no radio frequency heard in the area of the tracks) we also documented 1-2 additional kittens born spring 2003 and still alive in winter 2004.

Of the 16 kittens we found in summer 2003, we documented the following by April 2004: 6 confirmed alive, 7 confirmed dead, and 3 some evidence dead. Although we tried, we were not able to capture any of the 6 surviving kittens to fit them with radio-collars.

2004.-- In Spring 2004, 26 females from the releases in 1999, 2000 and 2003 had active radio-collars. Of these, we documented 18 possible mating pairs of lynx during breeding season. All 4 of the females that had kittens with them through winter 2003-04 bred again spring 2004, 2 with the same male they successfully bred with spring 2003. During May-June 2004 we found 11 dens and a total of 30 kittens (Table 6). At all dens the females appeared in excellent condition, as did the kittens. The kittens weighed from 250-770 grams. Three of the 11 females with kittens were from the 2003 releases (Table 6). Three additional litters were documented after denning season through either observation of a female lynx with kittens or snow-tracking females with kittens that were not one of the 11 females found on dens. From the size of the kittens they would have been born during the normal denning season in May or June. Nine additional kittens were observed from these litters for a total of 39 known kittens born in 2004. Two of these additional litters were documented from direct follow-ups to sighting made by the public and reported to CDOW.

Two females that had kittens in 2003 and reared at least part of their litters through March 2004, bred and had kittens again in 2004. Two of the litters documented by direct observation or snow-tracking are from females whose collars no longer work. Seven kittens born in 2004 were captured at 10-months of age and fitted with dual satellite/VHF collars. All 7 are alive and currently being monitored.

2005.-- In spring 2005 we had 34 females from the releases in 1999, 2000, 2003 and 2004 that had active radio-collars. We documented 23 possible mating pairs of lynx during breeding season. During May-June 2005 we visited 16 dens and found a total of 46 kittens (Table 7). At all dens the females appeared in excellent condition, as did the kittens. An additional female had a den we were not able to get to during May or June due to high water. Female BC03F03 was hit and killed on I70 on 5/19/2005. She had 2 fetuses in her uterus, so would have contributed to reproduction this year had she lived.

We weighed, photographed, PIT-tagged the kittens and recorded sex. We also took blood samples from the kittens for genetic work in an attempt to confirm paternity. While we were working with the kittens the females remained nearby, often remaining visible to us. The females generally continued a low growling vocalization the entire time we were at the den. In all cases, the female returned to the den site once we left the area.

All of the 2005 dens were scattered throughout the high elevation areas of Colorado, south of Interstate 70. Most of the dens were in Engelmann spruce/subalpine fir forests in areas of extensive downfall. Elevations ranged from 3117-3586 m. We weighed, photographed, and PIT-tagged the kittens, recorded sex and took hair samples from the kittens for genetic work in an attempt to confirm paternity. Four of the females would not leave the den until we reached out to pick up a kitten. While we were working with the kittens the females remained nearby, often remaining visible to us. The females generally continued a low growling vocalization the entire time we were at the den. In all cases, the female returned to the den site once we left the area.

One female, YK00F10 has had litters 3 years in a row. In 2003 she had 4 kittens and raised 2 through the winter. In 2004 she had 2 kittens and raised both through the winter, this year she had 4 kittens again. She has had all 3 litters in the same general area and has had the same mate for 3 years. Eight additional females had a second litter in Colorado this year. Three females from the 2004 releases had litters in 2005. This is the second year in a row we had females released the prior spring, find a territory and a mate within a year and produced live young. In reproduction season 2004 we had 3 females released in spring 2003 that did the same thing. Of those 3, 2 successfully raised at least part of their litters through winter 2005.

Den Sites.--A total of 33 dens have been found. All of the dens except one have been scattered throughout the high elevation areas of Colorado, south of I-70. One den was found in southeastern Wyoming, near the Colorado border. Dens were located on steep ($\bar{x}_{slope} = 29^{o}$), north-facing, high elevation ($\bar{x}$ = 3347 m) slopes (Figure 2). The dens were typically in Engelmann spruce/subalpine fir forests in areas of extensive downfall (Figures 3, 4, 5).

Recaptures

Two adult lynx were captured in 2001 for collar replacement. One lynx was captured in a tomahawk live-trap, the other was treed by hounds and then anesthetized using a jab pole. Five adult lynx were captured in 2002; 3 were treed by hounds and 2 were captured in padded leghold traps. In 2004, 1 lynx was captured with a Belisle snare and 6 other adult lynx were captured in box-traps. Trapping effort was substantially increased in winter and spring 2005 and 12 adult lynx were captured and re-collared. In addition, 7 kittens born in Colorado in 2004 were also captured and collared. All lynx captured in 2005 were caught in box-traps. All captured lynx were fitted with new Sirtrack ™ dual VHF/satellite collars.

Six adult lynx were captured from March 1999-June 30, 2005 because they were in poor body condition. Five of these lynx were successfully treated at the Frisco Creek Rehabilitation Center and re-released in the Core Research Area. One lynx, BC00F7, died from starvation and hypothermia. Two lynx were captured because they were in atypical habitat outside the state of Colorado. They were held at Frisco Creek Rehabilitation Center for a minimum of 3 weeks and re-released in the Core Research Area in Colorado. Prior to release these lynx were fitted with new Sirtrack ™ dual VHF/satellite collars.

HABITAT USE

Landscape-scale daytime habitat use was documented from 7421 aerial locations of lynx collected from February 1999-June 30, 2005. Throughout the year Engelmann spruce / subalpine fir was the dominant cover used by lynx. A mix of Engelmann spruce, subalpine fir and aspen (*Populus tremuloides*) was the second most common cover type used throughout the year. Various riparian and riparian-mix areas were the third most common cover type where lynx were found during the daytime flights. Use of Engelmann spruce-subalpine fir forests and Engelmann spruce-subalpine fir-aspen forests was similar throughout the year. There was a trend in increased use of riparian areas beginning in July, peaking in November, and dropping off December through June.

Site-scale habitat data collected from snow-tracking efforts indicate Engelmann spruce and subalpine fir were also the most common forest stands used by lynx for all activities during winter in southwestern Colorado. Comparisons were made among sites used for long beds, dens, travel and where they made kills. Little difference in aspect, mean slope and mean elevation were detected for 3 of the 4 site types including long beds, travel and kills where lynx typically use gentler slopes ($\bar{x} = 15.7^{o}$) at an mean elevation of 3173 m, and varying aspects with a slight preference for north-facing slopes (Figure 2).

Den sites however, were located at higher elevations ($\bar{x}$ = 3347 m), steeper slopes ($\bar{x}$ = 29^{o}) and more commonly on north-facing slopes (Figure 2).

Mean percent total overstory was higher for long bed and kill sites than travel or den sites (Figure 3). Engelmann spruce provided a mean of 35.87% overstory for kills and long beds, with travel sites averaging 28% and den sites having the lowest mean percent overstory of 23% (Figure 3). Mean percent subalpine fir or aspen overstory did not vary across use sites (Figure 3). Willow overstory was highly variable and no dens were located in willow overstory.

A total of 1841 site-scale habitat plots were completed in winter from December 2002 through April 2005. The most common understory species at all 3 height categories above the snow (low = 0-0.5m, medium = 0.51 - 1.0 m, high = 1.1 - 1.5 m) was Engelmann spruce, subalpine fir, willow (*Salix* spp.) and aspen (Figure 4). Various other species such as Ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), cottonwood (*Populus sargentii*), birch (*Betula* spp.) and others were also found in less than 5% of the habitat plots. If present, willow provided the greatest percent cover within a plot followed by Engelmann spruce, subalpine fir, aspen and coarse woody debris for long beds, kills and travel sites. Areas documented in willow used by lynx are typically on the edge of willow thickets as tracks are quickly lost within the thicket. Den sites had significantly higher percent understory cover for all three height categories. Understory at den sites was primarily made up of coarse woody debris (Figure 3).

The most common tree species documented in the site-scale habitat plots was Engelmann spruce Figure 5). Subalpine fir and aspen were also present in >35% of the plots. Most habitat plots were vegetated with trees of DBH < 6" (Figure 5). As DBH increased, percent occurrence decreased within the plot. Although decreasing in abundance as size increased, most lynx use sites had trees in each of the DBH categories, indicating mature forest stands except for dens. Den sites had a broad spectrum of Engelmann spruce tree sizes, including > 18” but no large subalpine fir or aspen trees. While Engelmann spruce and subalpine fir occurred in similar densities for kills, long beds and travel sites, den sites had twice the density of subalpine firs found at all other sites (Figure 5).

DIET AND HUNTING BEHAVIOR

Winter diet of lynx was documented through detection of kills found through snow-tracking. Prey species from failed and successful hunting attempts were identified by either tracks or remains. Scat analysis also provided information on foods consumed. A total of 400 kills were located from February 1999-April 2005. We collected 671 scat samples from February 1999-April 2004 that will be analyzed for content. In each winter, the most common prey item was snowshoe hare, followed by red squirrel (Table 8).

A comparison of percent overstory for successful and unsuccessful snowshoe hare chases indicated lynx were more successful at sites with slightly higher percent overstory, if the overstory species were Englemann spruce, subalpine fir or willow. Lynx were slightly less successful in areas of greater aspen overstory (Figure 6). This trend was repeated for percent understory at all 3 height categories (Figure 7) except that higher aspen understory improved hunting success. Higher density of Engelmann spruce and subalpine fir increased hunting success while increased aspen density decreased hunting success (Figure 8).

DISCUSSION

In an effort to establish a viable population of lynx in Colorado, CDOW initiated a reintroduction effort in 1997 with the first lynx released in winter 1999. From 1999 through spring 2004, 166 lynx were released in the Core Research Area. The reintroduction effort was augmented with the release of 37 additional animals in April 2005 and 1 in June 2005, bringing the total to 204 lynx reintroduced to southwestern Colorado.

Locations of each lynx were collected through aerial- or satellite-tracking to document movement patterns and to detect mortalities. Most lynx remain in the southwestern quarter of Colorado. Dispersal movement patterns for lynx released in 2000 and subsequent years were similar to those of lynx released in 1999. However, more animals released in 2000 and subsequent years remained within the Core Research Area than those released in 1999. This increased site fidelity may have been due to the presence of con-specifics in the area on release. Numerous travel corridors have been used repeatedly by more than 1 lynx. These travel corridors include the Cochetopa Hills area for northerly movements, the Rio Grande Reservoir-Silverton-Lizardhead Pass for movements to the west, and southerly movements down the east side of Wolf Creek Pass to the southeast to the Conejos River Valley. Lynx appear to remain faithful to an area during winter months, and exhibit more extensive movements away from these areas in the summer. Most lynx currently being tracked are within the Core Research Area. During the summer months, lynx were documented to make extensive movements away from their winter use areas. Extensive summer movements away from areas used throughout the rest of the year have been documented in native lynx in Wyoming and Montana (Squires and Laurion 1999). Human-caused mortality factors such as gunshot and vehicle collision are currently the highest causes of death.

Reproduction is critical to achieving a self-sustaining viable population of lynx in Colorado. Reproduction was first documented from the 2003 reproduction season and again in 2004 and 2005. Additional reproduction is likely to have occurred in females we are no longer tracking, and from Colorado born lynx that have not been collared. The dens we find are more representative of the minimum number of litters and kittens in a reproduction season. Live-births and over-winter survival of kittens are the first steps towards recruitment into the breeding population defined as when these Colorado-born lynx will produce offspring of their own. To achieve a viable population of lynx, enough kittens need to be recruited into the population to offset the mortality that occurs in that year and hopefully even exceed the mortality rate for an increasing population.

Mowat et al. (1999) suggest lynx and snowshoe hare select similar habitats except that hares select more dense stands than lynx. Very dense understory limits hunting success of the lynx and provides refugia for hares. Given the high proportion of snowshoe hare in the lynx diet in Colorado, we might then assume the habitats used by reintroduced lynx also depict areas where snowshoes hare are abundant and available for capture by lynx in Colorado. From both aerial locations taken throughout the year and from the site-scale habitat data collected in winter, the most common areas used by lynx are in stands of Engelmann spruce and subalpine fir. This is in contrast to adjacent areas of Ponderosa pine, pinyon juniper, aspen and oakbrush. The lack of lodgepole pine in the areas used by the lynx may be more reflective of the limited amount of lodgepole pine in southwestern Colorado, the Core Research Area, rather than avoidance of this tree species.

Hodges (1999) summarized habitats used by snowshoe hare from 15 studies as areas of dense understory cover from shrubs, stands that are densely stocked, and stands at ages where branches have more lateral cover. Species composition and stand age appears to be less correlated with hare habitat use than is understory structure (Hodges 1999). The stands need to be old enough to provide dense cover and browse for the hares and cover for the lynx. In winter, the cover/browse needs to be tall enough to still provide browse and cover in average snow depths. Hares also use riparian areas and mature forests with understory. Site-scale habitat use documented for lynx in Colorado indicate lynx are most commonly using areas with Engelmann spruce understory present from the snow line to at least 1.5 m above the snow. The mean percent understory cover within the habitat plots is typically less than 15% regardless of understory species. However, if the understory species is willow, percent understory cover is typically double that, with mean number of shrubs per plot approximately 80, far greater than for any other understory species.

In winter, hares browse on small diameter woody stems (<0.25"), bark and needles. In summer, hares shift their diet to include forbs, grasses, and other succulents as well as continuing to browse on woody stems. This shift in diet may express itself in seasonal shifts in habitat use, using more or denser coniferous cover in winter than in summer. The increased use of riparian areas by lynx in Colorado from July to November may reflect a seasonal shift in hare habitat use in Colorado. Major (1989) suggested lynx hunted the edge of dense riparian willow stands. The use of these edge habitats may allow lynx to hunt hares that live in habitats normally too dense to hunt effectively. The use of riparian areas and riparian-Engelmann spruce-subalpine fir and riparian-aspen mixes documented in Colorado may stem from a similar hunting strategy. However, too little is known about habitat use by hares in Colorado to test this hypothesis at this time.

Lynx also require sufficient denning habitat. Denning habitat has been described by Koehler (1990) and Mowat et al. (1999) as areas having dense downed trees, roots, or dense live vegetation. We found this to be in true in Colorado as well. In addition, the dens used by reintroduced lynx were at high elevation, steep north-facing slopes. All females that were documented with kittens denned in areas within their winter-use area.

Snow-tracking of released lynx provided information on hunting behavior and diet through documentation of kills, food caches, chases, and diet composition estimated through prey remains. Snow-tracking results indicate the primary winter prey species are snowshoe hare and red squirrel, with other mammals and birds forming a minor part of the winter diet. In winter, lynx reintroduced to Colorado appear to be feeding on their preferred prey species, snowshoe hare and red squirrel in similar proportions as those reported for northern lynx during lows in the snowshoe hare cycle (Aubry et al., 1999). Caution must be used in interpreting the proportion of identified kills. Such a proportion ignores other food items that are consumed in their entirety and thus are biased towards larger prey and may not accurately represent the proportion of smaller prey items, such as microtines, in lynx winter diet. Through snow-tracking we have evidence that lynx are mousing and several of the fresh carcasses have yielded small mammals in the gut on necropsy. The summer diet of lynx has been documented to include less snowshoe hare and more alternative prey than in winter (Mowat et al., 1999). All evidence suggests reintroduced lynx are finding adequate food resources.

SUMMARY

From results to date it can be concluded that CDOW has developed release protocols that ensure high initial post-release survival, and on an individual level lynx have demonstrated they can survive long-term in areas of Colorado. It has also been documented that reintroduced lynx could exhibit site fidelity, engage in breeding behavior and produce kittens. What is yet to be demonstrated is whether current conditions in Colorado can support the recruitment necessary to offset annual mortality for a population to sustain itself. Monitoring of reintroduced lynx will continue in an effort to document such viability.

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Table 1. Definitions of forest structure classes used to describe habitat sites (Thomas 1979).

Forest Structure	Class Definition
Grass/forb	The grass/forb stage is created naturally by a catastrophic event, such as wildfire, and is typified by the near complete absence of snags, litter or down material in the aspen and ponderosa pine types, or vice versa in the lodgepole or subalpine forest types.
Shrub/seedling	The shrub/seedling stage occurs when tree seedlings or shrubs grow up to 2.5 cm at diameter breast height (DBH), either naturally or artificially through planting.
Sapling/pole	The sapling/pole stage is a young stage where tree DBH's range from 2.5-17.5 cm with tree heights ranging 1.8-13.5 m. These trees are 5-100 years of age, depending on species and site condition.
Mature	The mature stage occurs when tree diameters reach a relatively large size (25-50 cm) and the trees are usually 90 or more years old. Forest stands begin to experience accelerated mortality from disease and insects.
Old-growth	The old-growth stage occurs when a mature stand is at advanced age (100 years for aspen or 200 years for spruce), is very slow growing, and has advanced degrees of disease, insects, snags, and down, dead material. An exception to this occurs in ponderosa pine and aspen types where these old-growth stands typically experience low densities of down dead material or snags.

Table 2. Lynx released in Colorado from February 1999 through June 30, 2005.

Year	Females	Males	TOTAL
1999	22	19	41
2000	35	20	55
2003	17	16	33
2004	17	20	37
2005	18	20	38
TOTAL	109	95	204

Table 3. Causes of death for adult lynx released into southwestern Colorado in 1999-2005 as of June30, 2005.

Cause of Death	Number of Mortalities
Unknown	22
Starvation	9
Hit by Vehicle	9
Shot	8
Probable Shot	6
Plague	4
Probable Predation	2
Probable Hit by Vehicle	2
Other Human Caused	2
Illness	1
Territorial Dispute	1
Total Mortalities	66

Table 4. Status of adult lynx reintroduced to Colorado as of June 30, 2005.

	Females	Males	Unknown	TOTALS
Released	109	95		204
Known Dead	40	25	1	66
Possible Alive	69	70		138
Missing	16	13		28[a]
Tracking	53	57		110

[a] 1 is unknown mortality

Table 5. Lynx reproduction documented in 2003.

Female	Release Year	Date Den Found	Number of Kittens		
			Females	Males	Total
BC00F8	2000	5/21/03	?	?	2
BC00F19	2000	5/26/03	1	1	2
YK00F16	2000	6/19/03	1	1	2
YK99F1	1999	6/10/03	2	1	3
YK00F19	2000	6/11/03	1	2	3
YK00F10	2000	5/31/03	2	2	4
		TOTAL	7	7	16

Table 6. Lynx reproduction documented in 2004.

Female ID	Release Year	Previous Litter	Date Den Found	Date Kittens Found	Number of Kittens		
					Females	Males	Total
YK00F2	2000		5/28/2004		3	1	4
AK00F2	2000		5/31/2004		2	1	3
YK00F1	2000		6/1/2004		3		3
YK00F15	2000		6/4/2004		1	2	3
BC00F14	2000		6/7/2004		1	2	3
BC00F18	2000		6/10/2004		4		4
YK00F10	2000		6/17/2004		1	1	2
BC03F02	2003		6/25/2004			2	2
BC03F10	2003		6/26/2004		2		2
BC03F09	2003		6/29/2004		1	1	2
YK00F7	2000		6/30/2004		1	1	2
YK99F1	1999			Dec 2004			2
Unknown				Sept 2004			4
Unknown				Feb 2005			3
TOTAL					19	11	39

Table 7. Lynx reproduction documented in 2005.

Female ID	Release year	Previous Litters	Date Den Found	Number of Kittens		
				Total	Males	Females
AK00F02	2000	2004	5/21/2005	3	2	1
YK00F15	2000	2004	5/28/2005	2	1	1
YK00F10	2000	2003, 2004	6/1/2005	4	2	2
YK00F11	2000		6/9/2005	2		2
YK00F01	2000	2004	6/10/2005	3	2	1
YK00F07	2000	2004	6/14/2005	3	1	2
BC00F18	2000	2004	6/24/2005	2	1	1
BC03F02	2003	2004	5/25/2005	2	1	1
BC03F01	2003		5/27/2005	4	2	2
QU03F06	2003		6/5/2005	3	3	
QU03F04	2003		6/14/2005	3	1	2
QU03F07	2003		6/16/2005	4	3	1
BC03F09	2003	2004	6/27/2005	2	1	1
BC03F10	2003	2004	-	?		
BC04F01	2004		6/11/2005	3	2	1
BC04F03	2004		6/19/2005	3		3
BC04F05	2004		6/23/2005	3	3	
TOTAL				46	25	21

Table 8. Number of kills found each winter field season through snow-tracking of lynx and percent composition of kills of the three primary prey species.

Field Season	n	Prey (%)			
		Snowshoe Hare	Red Squirrel	Cottontail	Other
1999	9	55.56	22.22	0	22.22
1999-2000	83	67.47	19.28	1.20	12.05
2000-2001	89	67.42	19.10	8.99	4.49
2001-2002	54	90.74	5.56	0	3.70
2002-2003	65	90.77	6.15	0	3.08
2003-2004	37	67.57	27.03	2.70	2.70
2004-2005	78	83.33	10.26	0	6.41

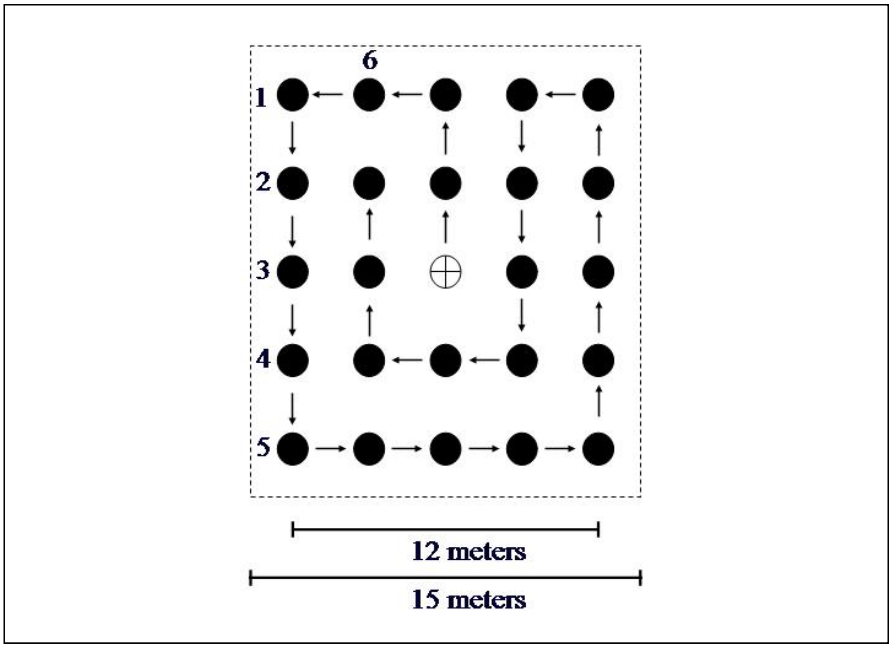

Figure 1. Design of site-scale habitat plot sampling plot. Each of the 25 points are 3 meters apart (the first 6 points are labeled 1-6). The object that triggered a habitat plot (e.g., kill) is the center point, depicted by the hollow circle. The actual pints encompass a 12 m x 12 m square but the understory and overstory data collected are influenced by vegetation occurring within a 15 m x 15 m square.

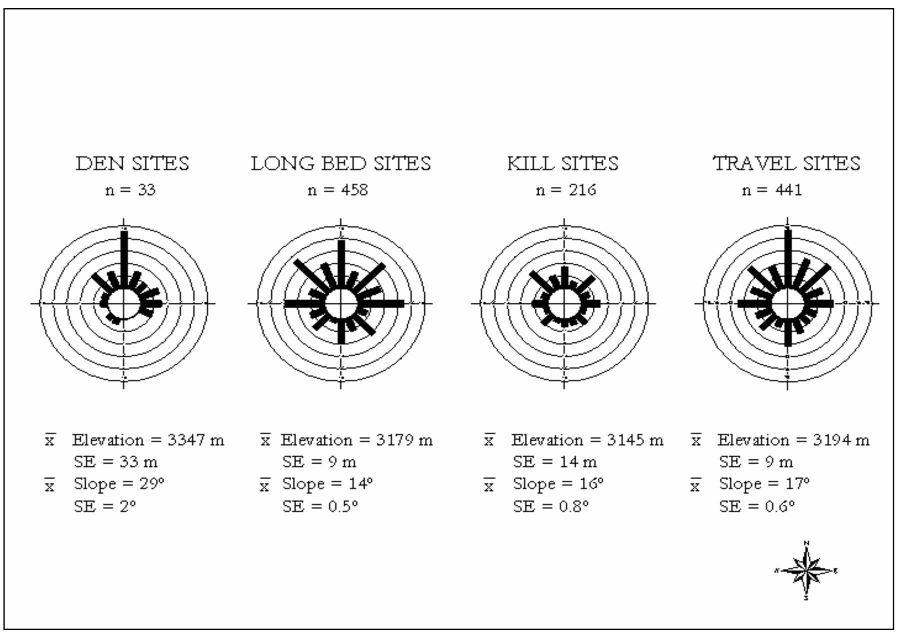

Figure 2. Frequency of aspect, mean elevation and SE and mean slope and SE for 4 lynx use sites; dens, long beds, kills and travel.

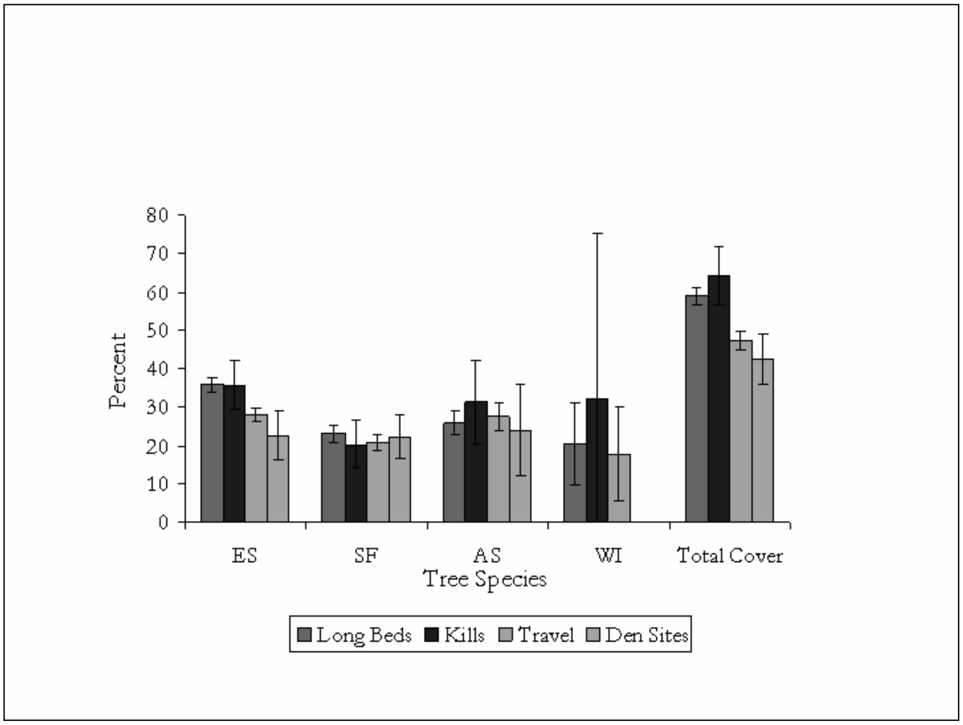

Figure 3. Mean percent overstory by tree species Engelmann spruce (ES), subalpine fir (SF), aspen (AS), willow (WI) and total cover for 4 different lynx use sites: long beds, kill sites, travel and den sites. Confidence intervals (95%) are depicted by error bars.

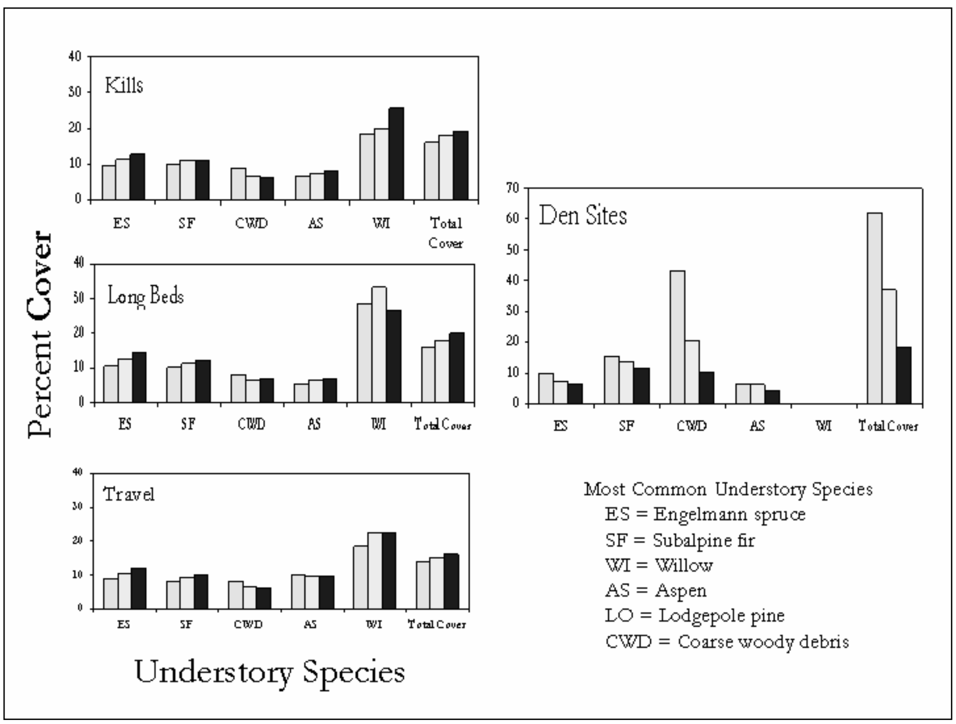

Figure 4. Mean percent understory by tree species Engelmann spruce (ES), subalpine fir (SF), coarse woody debris (CWD), aspen (AS), willow (WI) and total cover for 4 different lynx use sites: long beds, kill sites, travel and den sites.

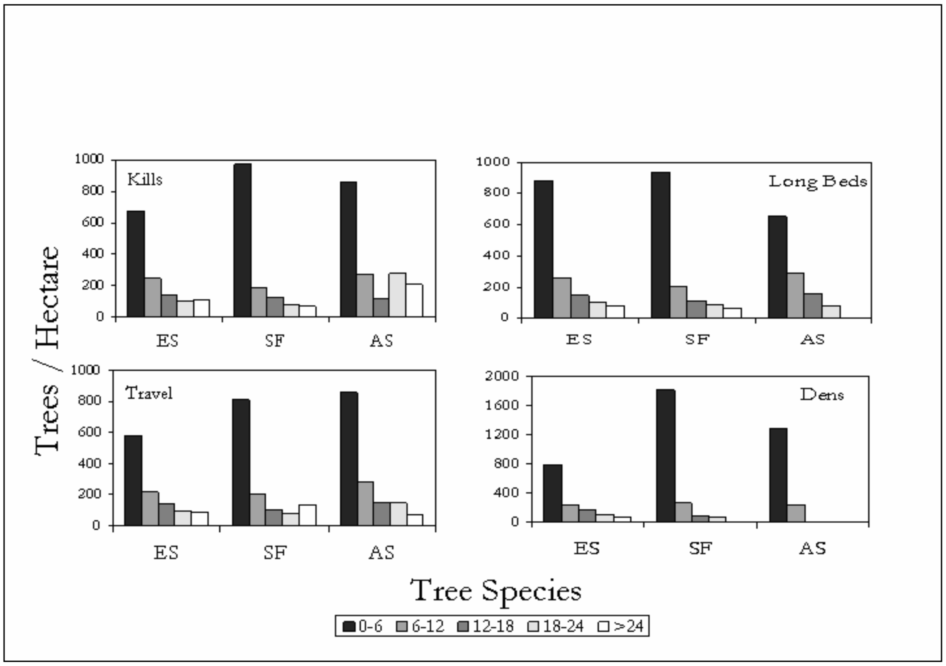

Figure 5. Mean tree density by species Engelmann spruce (ES), subalpine fir (SF) and aspen (AS) and dbh size class for 4 different lynx use sites.

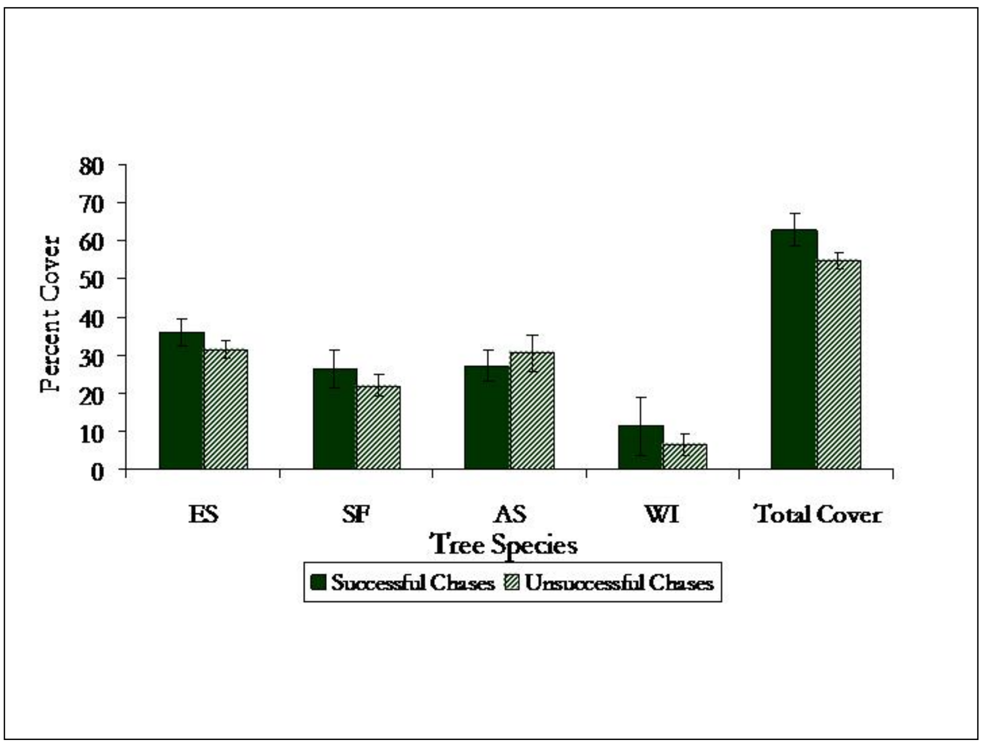

Figure 6. Mean percent overstory by tree species Engelmann spruce (ES), subalpine fir (SF), aspen (AS), willow (WI) and total cover for successful and unsuccessful snowshoe hare chases. Confidence intervals (95%) are depicted by error bars.

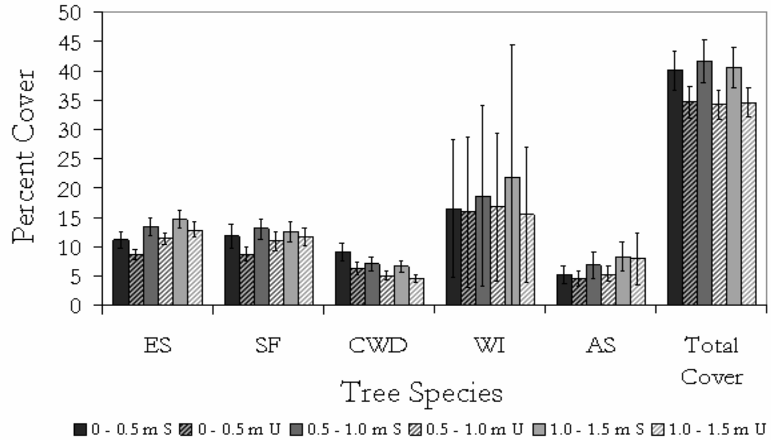

Figure 7. Mean percent understory by tree species Engelmann spruce (ES), subalpine fir (SF), aspen (AS), willow (WI) and total cover for 3 different understory height categories for successful and unsuccessful snowshoe hare chases. Confidence intervals (95%) are depicted by error bars.

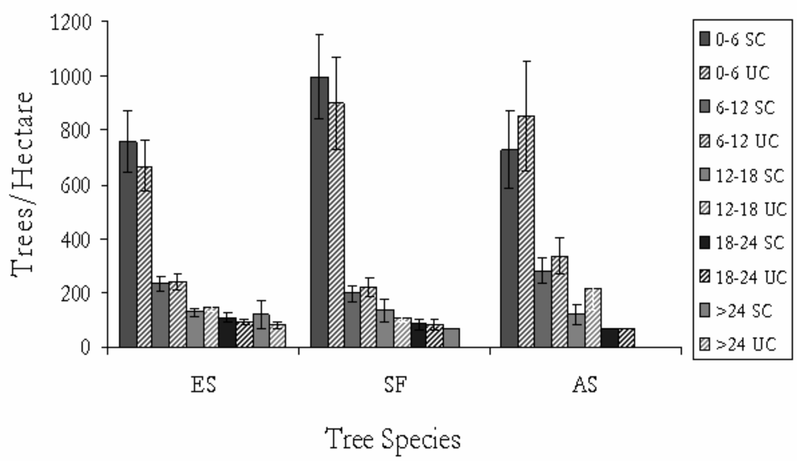

Figure 8. Mean tree density by species Engelmann spruce (ES), subalpine fir (SF) and aspen (AS) and 5 DBH size classes for successful chases (SC) and unsuccessful chases (UC) of snowshoe hare.

Colorado Division of Wildlife
July 2004 – June 2005

WILDLIFE RESEARCH REPORT

State of	Colorado	:	Division of Wildlife
Cost Center	3430	:	Mammals Research
Work Package	3001	:	Deer Conservation
Task No.	2	:	Pilot Evaluation of Winter Range Habitat Treatments on Mule Deer Fawn Over- Winter Survival
Federal Aid Project	W-185-R	:	

Period Covered: July 1, 2004 - June 30, 2005

Author: E.J. Bergman, C.J. Bishop, D.J. Freddy and G.C. White

Personnel: D. Baker, B. Banulis, M. Catanese, M. Cowardin, K. Crane, B. deVergie, K. Duckett, D. Hale, C. Harty, J. McMillan, R. Swygman, C. Tucker, S. Waters, B. Watkins, M. Zeaman, CDOW; L. Carpenter, WMI; and, project support provided by Federal Aid in Wildlife Restoration and the Mule Deer Foundation.

All information in this report is preliminary and subject to further evaluation. Information MAY NOT BE PUBLISHED OR QUOTED without permission of the author. Manipulation of these data beyond that contained in this report is discouraged.

ABSTRACT

A pilot study Program Narrative (Appendix I) study plan was developed to test the effects of winter range habitat treatments on mule deer fawn survival rates on the Uncompahgre Plateau in southwest Colorado. Data were collected in order to confirm logistical feasibility and to establish baseline estimates for survival and process variation of survival. Overwinter fawn survival in our study areas was very high, (BCSWA $\hat{S}$ = 0.84 (SE = .075), Sowbelly $\hat{S}$ = 0.96 (SE = .039), combined $\hat{S}$ = 0.90 (SE = .042). Data collected during this pilot study proved to be very informative and are useful in the development of a full research project study plan. Our initial objectives of assessing the logistical/financial feasibility of conducting field work in our chosen study areas were adequately addressed. Based on these results, we found that each method of capture was appropriate for the respective study areas. While helicopter net-gunning was not minimized due to unfavorable ground-based capture conditions, financial limitations in terms of captures costs were not broached. Despite remote location, monitoring of survival in one of our study areas was also adequately accomplished. Survival rate estimation from this study will also serve as baseline data for the estimation of process variation in survival during a full research project.

WILDLIFE RESEARCH REPORT

PILOT EVALUATION OF WINTER RANGE HABITAT TREATEMENTS ON OVER-WINTER MULE DEER FAWN SURVIVAL.

E.J. BERGMAN, C.J. BISHOP, D.J. FREDDY AND G.C. WHITE

P.N. OBJECTIVE

1. To conduct a one-year pilot study to assess the logistical feasibility of the proposed study herein and to gather preliminary data to improve a full research project's efficiency and experimental design.

SEGMENT OBJECTIVES

1. Prepare a pilot study Program Narrative and complete field work accordingly.

2. To collect survival data in 2 study areas to measure baseline survival rates and to learn about differences between study areas.

3. To establish baseline collection of survival data in order to produce reliable estimates of annual process variation necessitated by the longevity of a proposed full research project.

4. Begin work on a full research project Program narrative study plan.

5. Acquire necessary field equipment (radio collars, receivers and antennas) and additional funding for implementation of full research project.

INTRODUCTION

As with many wildlife species, mule deer populations tend to fluctuate such that there are noticeable differences between highs and lows. Several dramatic fluctuations have been observed since the turn of the 19th century (Connolly 1981, Gill 2001), with the most recent decline taking place during the 1990's (Unsworth et al. 1999). Wildlife managers' challenges are thus two-fold: understanding the underlying causes of population fluctuations and managing populations to dampen the effects of these fluctuations.

Recent research conducted by the Colorado Division of Wildlife has assessed the role of forage quality and quantity on over-winter fawn survival (Bishop et al. 2004). Using a treatment/control cross-over design, the impact of *ad libitum* pelleted food supplements as a surrogate for habitat improvement, was measured. The primary hypothesis behind this research concerned the interaction between predation and nutrition. If supplemental forage treatments improved over-winter fawn survival (i.e., if predation did not prevent an increase), then it could be concluded that over-winter nutrition was the limiting factor on population performance. As such, preliminary evidence suggests that nutrition enhancement treatments have increased fawn survival by as much as 20% (C.J. Bishop, personal communication). However, while this research elucidated some of the underlying processes in mule deer population regulation, it did not test the effectiveness of an acceptable management technique. Due to the undesirable effects of feeding wildlife (i.e. artificially elevating density, increased potential for disease transmission, cost and manpower), a more appropriate technique for delivering a high quality nutrition enhancement needs to be assessed.

The reasons for conducting this work as a pilot study were two-fold. First, we wished to determine if the capture and monitoring of deer in our chosen study areas was logistically feasible. One study area (Sowbelly/Tatum - see below) was known to have low deer densities and to be located in an area that was too remote to allow for ground based capture efforts. It was unknown if deer capture in this area, via helicopter net-gunning, would be economically feasible. The second study area (Billy Creek State Wildlife Area - see below) had high ungulate densities and was easily accessible from roads. Baited drop nets (Ramsey 1968, Schmidt et al. 1978, Bartmann et al. 1992) were the preferred method of capture in this area, however, the feasibility of using drop nets in this area needed to be evaluated in light of high elk densities. Drop netting in areas with large numbers of elk is not ideal because elk presence under a net, despite the number of deer, limits capture opportunities. The second primary reason for conducting a 1-year pilot study was to gather data and information to improve the design of future experimental research on the same topic. These data will allow us to improve our estimate of process variance in fawn survival, because fawn survival has been shown to vary significantly among areas and years (Unsworth et al. 1999, Bishop et al. 2005).

STUDY AREA

This pilot study was conducted on the Uncompahgre Plateau and in an adjacent valley in southwestern Colorado. The first study area, preliminarily identified as a high quality area, was located on Billy Creek State Wildlife Area (BCSWA - approximately 20km south of Montrose, CO). Over the past 15-30 years this area has received several habitat treatments that were intended to benefit mule deer. Additionally, BCSWA was in close proximity to agricultural lands that provided high quality, succulent forage immediately preceding the onset of winter and immediately following spring melt. The second study area, preliminarily identified as a low quality area, was located on Sowbelly and Tatum draws (Sowbelly - approximately 15km west of Delta, CO). This area was located in a segment of winter range that had not received any habitat treatments, was not in close proximity to agricultural lands and was in an advanced stage of pinyon-juniper succession (i.e. old growth trees, poor or non-existent understory and poor quality of shrub component).

METHODS

Twenty-five mule deer fawns were captured and radio-collared in each of the 2 study areas. At BCSWA we captured fawns with baited drop-nets (Ramsey 1968, Schmidt et al. 1978, Bartmann et al. 1992) and via helicopter net-gunning (Barrett et al. 1982, van Reenen 1982). At Sowbelly all fawns were captured via helicopter net-gunning. All captures occurred during December 2004. All capture and handling protocols were approved by CDOW ACUC committee (project number 09-2004).

On a daily basis, from December through May, we attempted to monitor the radioed fawns in order to document live/death status. Due to the remote location of Sowbelly, we found it necessary to supplement ground tracking with weekly aerial fixed-wing flights. Combined, these methods allowed us to calculate weekly survival rates and to accurately estimate the date and proximate causes of death. All fawns were located, via aerial telemetry, one time per month.

RESULTS AND DISCUSSION

Ground based capture efforts during this pilot study took place on 14 days between 7 December and 30 December. During this time, 36 nets were monitored and 9 drops were made, resulting in the capture of 12 individual fawns. No injuries or capture related mortalities occurred and 1 non-target animal (adult male elk) was captured and released without injury. Helicopter net-gunning took place on 3 consecutive days between 31 December and 2 January. Capture related injuries during net-gunning were limited to 1 animal, which was later euthanized.

Based on these results, we found that each method of capture was appropriate for the respective study areas. However, ground based capture efforts (confined to BCSWA) were not as efficient as originally hoped. We attribute this to mild winter conditions and the abundance of available native vegetation, as well as intermittent patches of green exotic forage. We suspect that under typical winter conditions (i.e. complete snow coverage of the study area) our efforts to bait deer into capture sites would have been more effective. Despite our poor efficiency, ground based capture efforts saved approximately $6,000. Our initial concern in regards to the cost of helicopter net-gunning in low density areas was not realized. The minimum sample size requirement of 25 fawns was met in 1.5 days of capture effort and all capture occurred at the per animal rate.

Direct capture related injury was low (injury occurred in 1 of 52 animals, <2%). No post hoc capture related injury or mortality was observed in either study area, indicating that potential bias resulting from multiple capture methods was not detectable. However, potential bias does exist due to the fact that the period of time needed to capture animals in two areas via multiple methods was quite long. In order to compare survival rates between the two areas, rates couldn't be calculated until minimum sample size requirements had been met in each area.

As expected based on deer density, differences in the size of winter range between the 2 study areas was very dramatic. The observed winter range size for radio marked deer on BCSWA was ~8.7km^2. The observed winter range size for radio marked deer on Sowbelly was 140 km^2. Despite these differences, overwinter fawn survival in both study areas was very high. The measured survival rate for BCSWA, the lowest for both areas, was $\hat{S} = 0.84$ (SE = .075) (Table 1 and Fig. 1a). Survival at Sowbelly, hypothesized to be an area that experiences lower survival, was $\hat{S} = 0.96$ (SE = .039) (Table 1 and Fig. 1b). Across the Uncompahgre Plateau, survival rates ($\hat{S} = 0.90$, SE = .042) (Fig. 1c) were much higher than rates reported in the literature (B. Banulis, personal communication, Unsworth et al. 1999).

SUMMARY

Data collected during this pilot study proved to be very informative and are useful in the development of a full research project study plan. Our initial objectives of assessing the logistical/financial feasibility of conducting field work in our chosen study areas were adequately addressed. While ground based capture efforts in BCSWA were not as efficient as initially hoped, we found that the more pressing concern of high elk density was not a limiting factor. To further reduce potential conflict concerning elk in and around deer drop nets, starting capture operations at an earlier date, prior to elk movement down onto BCSWA, would be prudent. Helicopter net-gunning efficiency in Sowbelly was also assessed. Capture was completed after 1.5 days of effort, demonstrating that despite low densities, utilization of this area in further research efforts is warranted. Despite remote location, monitoring of survival in Sowbelly was also adequately accomplished. Weekly survival rates were measured and it is believed that the addition of future study areas in the general proximity of Sowbelly during a full research project would not prove to be cost or logistically prohibitive. Survival rate estimation from this study will also serve as baseline data for the estimation of process variation in survival during a full research project.

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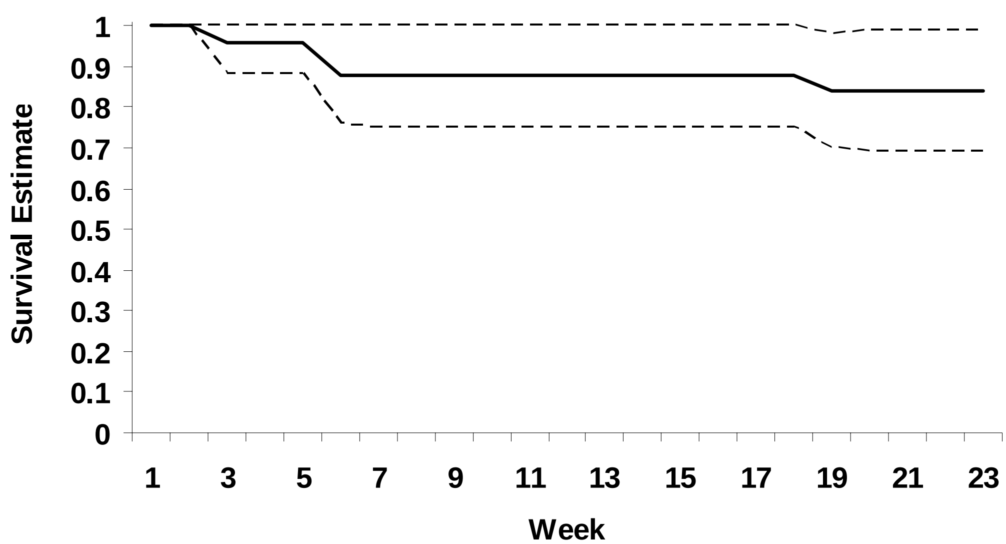
Prepared by______________________________
Eric J. Bergman, Wildlife Researcher

Table 1. Causes of over-winter mortality for mule deer fawns from two pilot study areas on the Uncompahgre Plateau, southwest Colorado.

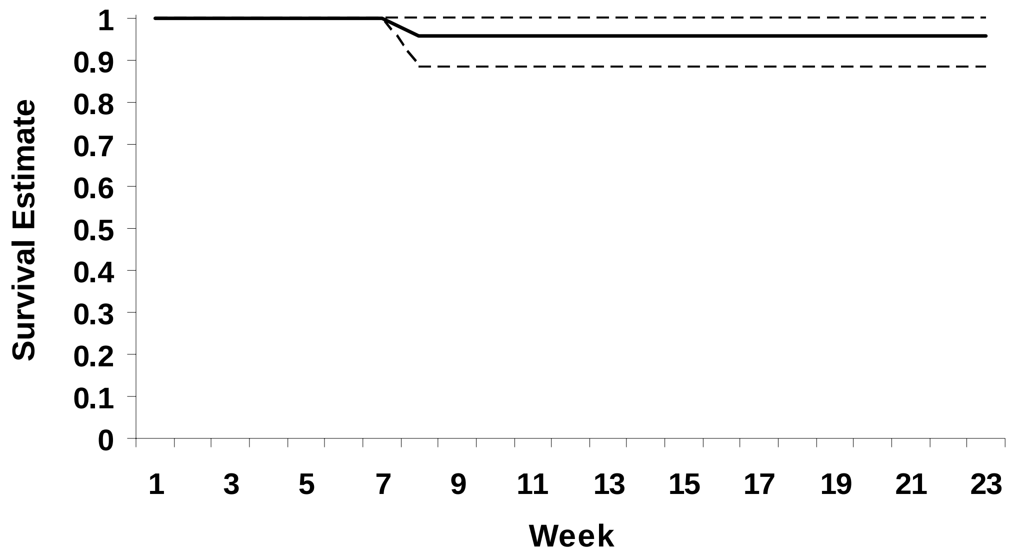
	Cause of Death			
Study Area	Felid	Canid	Sick/Starve	Unk
BCSWA	4	1	0	0
Sowbelly	0	0	0	1

Figure 1. Estimated overwinter mule deer fawn survival curves, with 95% confidence intervals, for pilot research conducted on Billy Creek State Wildlife Area (1a), Sowbelly and Tatum draws on the Uncompahgre Plateau (1b) and combined for the two areas (1c). Survival estimates (solid lines) and confidence intervals (dashed lines) were measured between late-December and mid-June.

1a



1b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Survival Estimate
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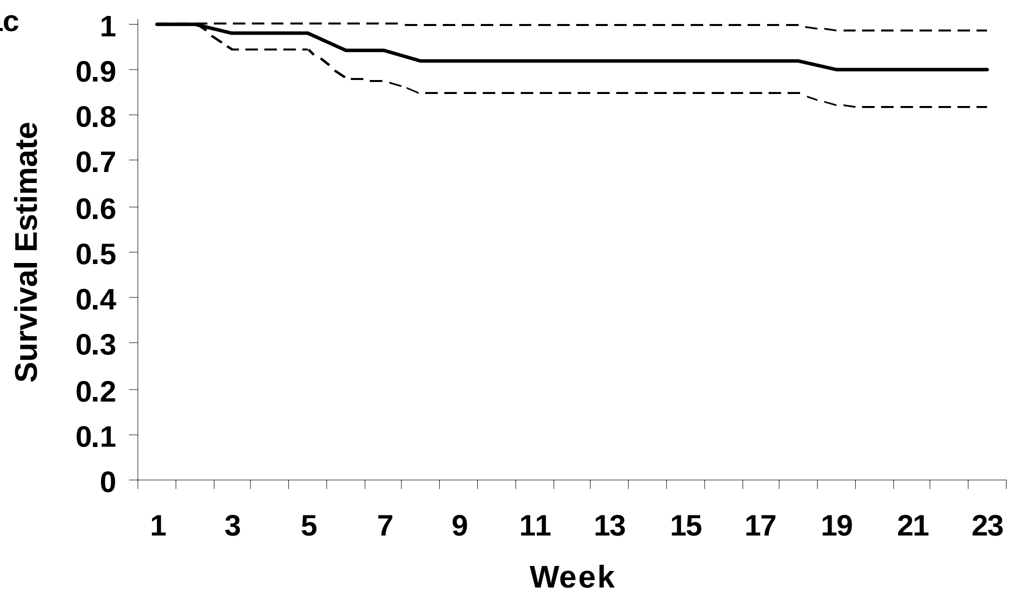
Week

1c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APPENDIX I

PROGRAM NARRATIVE STUDY PLAN FOR MAMMALS RESEARCH

PILOT EVALUATION OF WINTER RANGE HABITAT TREATEMENTS ON OVER-WINTER MULE DEER FAWN SURVIVAL.

A pilot study proposal submitted by:
E.J. Bergman, Colorado Division of Wildlife
C.J. Bishop, Colorado Division of Wildlife
G.C. White, Colorado State University
D.J. Freddy, Colorado Division of Wildlife

A. Need

As with many wildlife species, mule deer populations tend to fluctuate such that there are noticeable differences between highs and lows. Several dramatic fluctuations have been observed since the turn of the 19th century (Connolly1981, Gill 2001), with the most recent decline taking place during the 1990's (Unsworth et al. 1999). Wildlife managers' challenges are thus two-fold: understanding the underlying causes of population fluctuations and managing populations to dampen the effects of these fluctuations. As a result of these objectives and challenges, considerable energy and money has been invested in assessing the role of habitat quality on mule deer population performance.

Recent research conducted by the Colorado Division of Wildlife has assessed the role of forage quality and quantity on over-winter fawn survival (Bishop et al. 2004). Using a treatment/control cross-over design, the impact of *ad libitum* pelleted food supplements as a surrogate for habitat improvement, was measured. The primary hypothesis behind this research concerned the interaction between predation and nutrition. If supplemental forage treatments improved over-winter fawn survival (i.e., if predation did not prevent an increase), then it could be concluded that over-winter nutrition was the limiting factor on population performance. As such, preliminary evidence suggests that nutrition enhancement treatments have increased fawn survival by as much as 20% (C.J. Bishop, personal communication). However, while this research effectively elucidated some of the underlying processes in mule deer population regulation, it did not test the effectiveness of an acceptable management technique. Due to the undesirable effects of feeding wildlife (i.e. artificially elevating density, increased potential for disease transmission, cost and manpower), a more appropriate technique for delivering a high quality nutrition enhancement needs to be assessed.

Over the past 40 years land management agencies (BLM and USFS) have conducted habitat treatments, many of which have been driven by the desire to improve mule deer habitat. During the next five winters, we plan to quantify the impact of these treatments on mule deer population performance. We are proposing to measure fawn survival on a series of randomly selected study areas

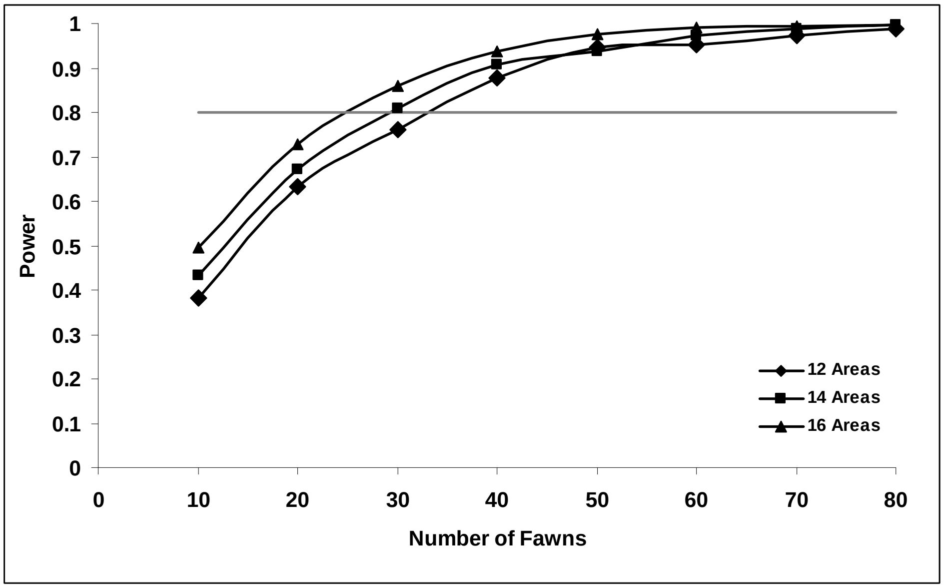

Figure 1. Expected power, based on simulation, of detecting a 20% (d = 0.20) difference in fawn survival across study areas at an α-level of 0.10.

Year	Unit A	Unit B	Unit C	Unit D	Unit E
Pilot	Control L				Control H
1	Control L	Random 1	Random 2	Random 3	Control H
2	Control L	Random 4	Random 5	Random 6	Control H
3	Control L	Random 7	Random 8	Random 9	Control H
4	Control L	Random 10	Random 11	Random 12	Control H

Figure 2**.** Schematic representation of study units and their allocation across years. The pilot study will consist of measuring fawn survival in a low quality control (Control L) and a high quality control (Control H). During subsequent years, fawn survival will continue to be measured on each of the controls to provide an estimate of the temporal process variance, and to adjust survival rates measured on the randomly selected sites for winter-to-winter differences.

that cover a range of habitat treatment quality from low to high. Power analyses (α = 0.10, β = 0.80) indicate that to detect a 20% change (d = 0.20) in survival, 25 fawns will need to be marked in each of 16 study areas (Fig. 1). Due to the logistical infeasibility of accomplishing this during one winter, we are proposing to measure fawn survival over 5 winters. However, spreading the design over multiple years confounds winter-to-winter variation in fawn survival with habitat effects. Therefore, we will measure fawn survival on 2 control areas during all years of the study to adjust for winter-to-winter effects. We propose measuring over-winter fawn survival in 2 control areas (2004 – 2008) and 3 randomly selected areas of variable habitat quality each year (2005 – 2008) (Fig. 2). We will measure fawn survival only in the control areas during the first winter as a pilot effort to obtain baseline data and gain experience with the logistics of this experiment.

The reasons for conducting the first winter of data collection as a pilot study are three-fold:
1) One of the control study areas is located in a low quality area (see Methods), an area that has never received habitat treatments and is not close in proximity to agricultural lands. We do not know if deer densities are high enough to capture a sample of 25 fawns in the area. Due to the remote location of this control area, helicopter net-gunning (Barrett et al. 1982, van Reenen 1982) is the only feasible capture technique. Helicopter net-gunning can become cost prohibitive in low density areas, emphasizing the need to test this approach prior to committing to four additional years. The high quality control area is defined by extensive exposure to habitat treatments as well as close proximity to agricultural fields (hay, alfalfa and/or grass), resulting in high densities of both deer and elk from these beneficial conditions. Because the high quality control area is easily accessible from roads and has high densities of deer, baited drop nets are the preferred method of capture. However, the feasibility and efficiency of drop nets need to be evaluated in light of high elk densities. Drop netting in areas with large numbers of elk is not ideal because elk presence under a net, despite the number of deer, limits capture opportunities. By assessing these potential problems through a pilot study, we will improve the efficiency and design of the full research study in later years.
2) We wish to collect one year of data in our control areas before instituting a monitoring program in the treatment areas in order to improve our estimate of process variance in fawn survival.
3) We will gain insight as to how much fawn survival varies between extremes in habitat quality. The final reason for conducting a pilot study prior to instituting a full study pertains to our ability to assess habitat quality as it relates to habitat improvements and mule deer winter range. From the perspective of this study, there are three primary components to habitat quality: 1) availability of native forage, 2) availability of agriculturally based forage, and 3) overall structure of habitat. By conducting the initial year of data collection as a pilot, we will be able to identify and rank study areas according to these criteria. While detailed methodologies have not been developed, the general approach will be to: 1) grossly quantify the total biomass of native forage using existing information on mule deer forage selection (Bartmann 1983), 2) compute the total amount and proximity of land devoted to agricultural practices, per study area, based on existing GIS data, and 3) rely on a panel of mule deer experts to provide a ranking of study areas based on vegetation complex, availability of cover and overall habitat structure. Information collected through these three steps will be merged, allowing a general ranking of each study area into habitat quality. Rankings will serve no purpose within the study other than to provide an efficient mechanism for stratifying sampling, such that a gradient of poor to high quality study areas can be sampled each year.

B. Objective

The specific objectives for our pilot study are: 1) determine if deer density is high enough in our low quality control study area to allow for the capture of 25 mule deer fawns, 2) determine if elk density in the high quality control study area (and if elk affinity for bait under drop nets) is too high to efficiently capture 25 mule deer fawns with baited drop nets, and 3) design and implement habitat assessment techniques that will allow us to segregate randomly selected study areas based on habitat quality, the impact of previous habitat treatments, and the proximity to agricultural lands.

C. Expected Results

We wish to determine if our proposed capture techniques are appropriate for the conditions under which we will be working. The null hypothesis for this research project is that fawn survival does not vary between study areas and therefore habitat treatment/improvement efforts do not enhance over-winter fawn survival. The alternative hypothesis is that habitat treatments improve survival and a general increase in fawn survival should be observed as habitat quality increases. This pilot study is designed to measure fawn survival under optimal and poor habitat conditions. The pilot study will provide preliminary estimates of the difference in fawn survival between high and low quality habitat.

Alternative approaches for quantifying the effectiveness of habitat treatments on fawn survival, including a treatment/control design which experimentally tests this question, have been discussed. However, due to numerous logistical limitations, a feasible field experiment incorporating random selection and replication of treatment and control areas isn't achievable. A quasi-experiment incorporating a single treatment and control area where fawn survival would be measured in each area before and after administration of a treatment in the treatment area was designed and evaluated. While this study may have been logistically feasible, lack of replication would have limited inference, and the study would have been subject to the various problems associated with having only one treatment and control area (e.g. an unplanned fire in the control area would nullify study results). Further, a time frame of >5 years would be required to fully evaluate the impact of the imposed habitat treatment. We concluded that the design described in this study plan is the most effective approach to evaluate the effects of habitat treatments across a large landscape given the inherent logistical constraints of adequate replication and duration of the study.

D. Approach

1. Procedures

A. Capture and Handling Methods: Twenty-five mule deer fawns will be captured and radio-collared in each of the 2 control study areas. In the high quality control, we will attempt to capture all fawns with baited drop-nets (Ramsey 1968, Schmidt et al. 1978, Bartmann et al. 1992). If needed, helicopter net-gunning will be used to complete the necessary sample in the high quality control. In the low quality control, all fawns will be captured via helicopter net-gunning (Barrett et al. 1982, van Reenen 1982). The confounding of area and capture methods should not be a problem, because White and Bartmann (1994) found no significant difference in survival of fawns 2 and 4 weeks after capture by these 2 methods for samples of 86 and 79 fawns. Captures will occur in December 2004.

B. Survival Monitoring: On a daily basis, from December through May, we will monitor the radioed fawns in order to document live/death status. This will allow us to determine accurately the date of death and estimate the proximate cause of death.

2. Use of Pilot Results

We will use the data collected during this research to evaluate preliminarily the expected magnitude of difference in fawn survival between areas with high quality habitat improvements and low quality areas with no habitat improvements. Power analyses ($\alpha = 0.10$, $\beta = 0.80$) indicate that similar measurements will be needed from 12 additional study areas during the next 4 winters in order to detect whether or not a 0.20 difference in fawn survival exists between low and high quality areas. Pilot data will be used to determine if such a study design is useful and feasible, and will provide evidence of whether our proposed capture methods will achieve the necessary sample sizes during the next four winters.

E. Location of Work

This pilot study will be conducted on the Uncompahgre Plateau and adjacent valleys in southwestern Colorado. The proposed high quality control area is located on Billy Creek State Wildlife Area (approximately 20km south of Montrose, CO). This area has received and continues to receive habitat treatments that are intended to benefit mule deer. Additionally, the high qualtiy control is in relatively close proximity to agricultural lands that provide high quality, succulent forage immediately preceding the onset of winter and immediately following spring melt. The proposed low quality control area is located on Sowbelly and Tatum draws (approximately 15km west of Delta, CO). This area is located in a segment of winter range that has not received any habitat treatments, is not in close proximity to agricultural lands and is in an advanced stage of pinyon-juniper succession (i.e. old growth trees, poor or non-existent understory and low frequency of shrub component).

F. Schedule and Work Assigned To

December 2004..Capture and deploy 25 VHF (172-174 MHZ frequency band) radio collars, fitted with growth/time deteriorating release mechanism, on fawns in each of two control study areas
December 2004 - May 2005.................................Monitor fawns for survival and retrieve collars as necessary
April 2005 - July 2005...Conduct habitat quality assessments on random study areas
July 2005 - August 2005......................................Produce preliminary results on the effectiveness of capture techniques and use pilot data to refine full-scale study plan as warranted.
October 2005..Retrieve collars that have dropped off fawns

G. Resource Requirements

Salaries of permanent and temporary employees, as well as other logistical costs (vehicles and flights) will be covered by existing game cash funds in the CDOW mule deer research and other CDOW programs. Expenditures specific to this study will include:

$11,200...50 radio collars
$15,000...Approximate costs for helicopter net-gunning 25 mule deer fawns
$800..Radio telemetry receivers
$300..Miscellaneous drop net equipment
$100..Bait for drop netting

$27,400...Total

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Colorado Division of Wildlife
July 2004 – June 2005

WILDLIFE RESEARCH REPORT

State of	Colorado	:	Division of Wildlife
Cost Center	3430	:	Mammals Research
Work Package	3001	:	Deer Conservation
Task No.	4	:	Effect of Nutrition and Habitat Enhancements on Mule Deer Recruitment and Survival Rate
Federal Aid Project:	W-185-R	:	

Period Covered: July 1, 2004 - June 30, 2005

Authors: C. J. Bishop, G. C. White, D. J. Freddy, and B. E. Watkins

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ABSTRACT

We measured mule deer (*Odocoileus hemionus*) population parameters in response to a nutrition enhancement treatment to evaluate the relative importance of habitat quality as a limiting factor of mule deer in western Colorado. During November 2000 – June 2004, we captured and radio-collared 810 individual mule deer evenly distributed among treatment and control units on the Uncompahgre Plateau in southwest Colorado. Our sample included 293 adult females, 154 of which received vaginal implant transmitters (VITs), 241 6-month-old fawns, and 276 newborn fawns born from either treatment or control adult does. We enhanced the nutrition of deer in the treatment unit by providing a safe, pelleted supplemental feed on a daily basis from December through April each winter. The treatment unit during winters 2000–01 and 2001–02 became the control unit during winters 2002–03 and 2003–04, and vice versa. Thus, the treatment effect was replicated across each experimental unit. Early winter fawn:doe ratios were measured using helicopter and ground classification surveys the year following treatment delivery to determine whether fawn production and survival increased as a result of enhanced nutrition of adult females. During winters 2001–02 through 2003–04, we measured pregnancy rates, fetus rates, and body condition of treatment and control adult does using ultrasonography. We measured fetus survival and neonate survival by using VITs to help locate and radio-collar newborn fawns born from treatment

and control does. We also measured overwinter fawn survival rates in response to the treatment. Estimated percent body fat of adult does during late February and early March, 2002–04, was higher ($F_{1, 148}$ = 153.41, $P < 0.001$) for treatment deer (9.8%, SE = 0.36, $n = 78$) than control deer (4.3%, SE = 0.26, $n = 76$). Serum thyroid hormone concentrations, measured only in 2003 and 2004, were higher in treatment does than control does ($F_{4, 108} = 46.59$, $P < 0.001$). Pregnancy and fetus rates were similar among treatment and control does. The pregnancy rate of adult does was 0.95 (SE = 0.036, $n = 38$) and the fetus rate was 1.80 fetuses/doe (SE = 0.10, $n = 36$) during 2002. Rates were similar in 2003, where we measured a pregnancy rate of 0.92 (SE = 0.034, $n = 63$) and a fetus rate of 1.74 fetuses/doe (SE = 0.069, $n = 50$) which included 5 yearlings (the fetus rate excluding yearlings was 1.82 fetuses/doe, SE = 0.066, $n = 45$). In 2004, pregnancy rate was 0.94 (SE = 0.029, $n = 66$) and fetus rate was 1.97 fetuses/doe (SE = 0.053, $n = 60$), which included 4 yearlings (fetus rate excluding yearlings was 2.00 fetuses/doe, SE = 0.051, $n = 56$). Based on multiple early winter age classification surveys, we lacked evidence to determine whether the winter nutrition enhancement treatment had any effect on neonatal production and survival during 2001, which provided additional incentive to directly measure fetus and neonate survival. During 2002–2004, fetus-neonate survival from 1 March–15 December was higher ($\chi^2_1 = 3.846$, $P = 0.050$) for treatment fawns ($S(t) = 0.528$, SE = 0.027) than control fawns ($S(t) = 0.401$, SE = 0.025). Survival data coupled with early winter age classification surveys provided evidence the nutrition enhancement treatment increased December fawn recruitment during 2002–2004. During 15 December–15 June, 2001–2004, the overwinter survival rate of fawns was greater ($\chi^2_1 = 18.781$, $P < 0.001$) in the treatment unit ($S(t) = 0.895$, SE = 0.029) than in the control unit ($S(t) = 0.655$, SE = 0.044). Using a staggered entry survival process with data combined over years, survival of treatment fetuses to 1 year of age ($S(t) = 0.458$, SE = 0.031) was 0.18 higher ($\chi^2_1 = 13.20$, $P < 0.001$) than survival of control fetuses to 1 year of age ($S(t) = 0.276$, SE = 0.026). The finite rate of population increase, λ, based on our measurements of treatment population parameters was 1.20, which would cause the deer population to double in approximately 4 years. The finite rate of increase calculated from control deer was 1.04, indicating a stable or slightly increasing population. The nutrition enhancement treatment therefore had a dramatic effect on deer population performance, indicating habitat quality was ultimately limiting the population. Our results provide a foundation for focusing deer management efforts on improving habitat quality in western Colorado pinyon-juniper (*Pinus edulis*-*Juniperus osteosperma*) ecosystems with corresponding research efforts to quantify the effects of habitat manipulations on deer performance.

WILDLIFE RESEARCH REPORT

EFFECT OF NUTRITION AND HABITAT ENHANCEMENTS ON MULE DEER RECRUITMENT AND SURVIVAL RATES

CHAD J. BISHOP, GARY C. WHITE, DAVID J. FREDDY, AND BRUCE E. WATKINS

P. N. OBJECTIVES

1. To determine experimentally whether enhancing mule deer nutrition during winter and early spring via supplementation increases fetus survival, neonate survival, overwinter fawn survival, or ultimately, population productivity.
2. To determine experimentally to what extent habitat treatments replicate the effect of enhanced nutrition from supplemental feeding.

SEGMENT OBJECTIVES

1. Radio-monitor and measure survival of the sample of radio-collared mule deer adult does and fawns.
2. Measure early winter fawn:doe ratios using both aerial helicopter surveys and ground classifications of deer groups associated with radio-collared adult does.
3. Summarize and analyze data and publish information in an annual Job Progress Report.
4. Complete a peer-reviewed manuscript for publication in a scientific journal pertaining to the effectiveness of vaginal implant transmitters for capturing mule deer neonates exclusively from radio-marked adult does (Appendix I).

INTRODUCTION

Mule deer (*Odocoileus hemionus*) numbers apparently declined during the 1990s throughout much of the West, and have clearly decreased since the peak population levels documented during the 1940s–1960s (Unsworth et al. 1999, Gill et al. 2001). Biologists and sportsmen alike have concerns as to what factors may be responsible for declining population trends. Although previous and current research indicates multiple interacting factors are responsible, habitat and predation have received the focus of attention. A number of studies have evaluated whether predator control increases deer survival, yet results are highly variable (Connolly 1981, Ballard et al. 2001). Together, predator control studies with adequate rigor and statistical power indicate predation effects on mule deer are variable as a result of time-specific and site-specific factors. Studies which have demonstrated deer population responses to predator control treatments have failed to determine whether predation is ultimately more limiting than habitat when considering long term population changes. Numerous research studies have evaluated mule deer habitat quality, but virtually no studies have documented population responses to habitat improvements. In many areas where declining deer numbers are of concern, predation is common yet habitat quality appears to have declined. The question remains as to whether predation, habitat, or some other factor is more limiting to mule deer in these situations, and whether habitat quality can be improved for the benefit of deer. It may also be that no single factor is responsible for observed deer declines, and a more comprehensive understanding of multi-factor interactions is needed.

We designed and implemented a field experiment where we measured deer population responses to a nutrition enhancement treatment to further understand the causative factors underlying observed deer population dynamics. We conducted the study on the Uncompahgre Plateau in southwest Colorado, where several predator species were present in abundant numbers: coyotes (*Canis latrans*), mountain lions (*Felis concolor*), and bears (*Ursus americanus*). In addition to predation, myriad diseases in combination have proximately affected survival of the Uncompahgre deer population (Pojar and Bowden

2004, B. E. Watkins, Colorado Division of Wildlife, unpublished data). Predator numbers were not manipulated in any manner during the course of the study. All factors were left constant with the exception of deer nutrition. Deer nutrition was enhanced by providing supplemental feed to deer occupying a treatment area during winter. We measured December fawn recruitment and overwinter fawn survival in response to the treatment to determine whether deer nutrition was ultimately more limiting than predation or disease. The second phase of the research will incorporate habitat manipulation treatments. The treatments will consist of prescribed fire or mechanical techniques to set back succession of pinyon-juniper (*Pinus edulis*-*Juniperus osteosperma*) habitat in an effort to improve the vigor and quality of winter habitat for mule deer. Deer population responses will be measured in relation to the habitat manipulations in the same manner as the supplemental feed. Thus, the experiment evaluates whether nutritional quality of winter range habitat is ultimately more limiting than other factors in a late-seral pinyon-juniper and sagebrush (*Artemisia* spp.) landscape, and if so, whether habitat can be effectively improved for mule deer. The results advance our understanding of multi-factor interactions, with direct implications for mule deer management.

STUDY AREA

We non-randomly selected two experimental units (A–B) within mule deer winter range on the Uncompahgre Plateau (Figure 1) to facilitate a cross-over experimental design for evaluating the effects of enhanced deer nutrition during winter on annual population performance. We used the following criteria to select experimental units:

1.) Deer densities (≥80 deer/mi^2): we selected areas where deer densities were sufficient to meet sample size requirements within the experimental unit, while simultaneously selecting areas that would require feeding no more than 600–800 animals during a normal winter;
2.) Buffer zones: we selected areas such that experimental units would be separated by several miles of non-treatment area (buffers) to prevent deer from occupying more than one experimental unit;
3.) Similarity: we selected areas that comprised relatively similar habitat complexes and deer densities that were representative of the overall area;
4.) Elk populations: we selected areas in an effort to minimize the number of elk present during normal winters.

Units A and B received the nutrition enhancement treatment in a cross-over experimental design to address P.N. Objective 1. Unit A served as the treatment unit, while Unit B served as the control, for the first 2 winters of research (2000 – 2002). During winters 2002–03 and 2003–04, Unit B received the treatment while Unit A served as the control. Upon completion of P.N. Objective 1, additional winter range experimental units will be used to conduct phase 2 of the research, or P.N. Objective 2. Habitat in treatment units will be manipulated to set back plant succession, while habitat in control units will remain unchanged throughout the experiment.

Experimental units A and B were defined as follows (Figures 2 and 3):

(1) Experimental unit A included the Colona Tract of the Billy Creek State Wildlife Area and adjacent land, located approximately 13 km south of Montrose, CO adjacent to U.S. Hwy 550 South. The experimental unit was located within the Colona USGS 7.5 Minute Quadrangle, and roughly included the polygon defined by the following Zone 13 UTM coordinates: (1) 254000 E, 4250200 N; (2) 252700 E, 4249400 N; (3) 254700 E, 4245600 N; and (4) 256200 E, 4246600 N.
(2) Experimental unit B included Shavano Valley and adjacent land extending west to the Dry Creek Rim. Shavano Valley is located approximately 13 km west of Montrose, CO. The experimental unit was located within the Dry Creek Basin and Montrose West Quadrangles (USGS 7.5 Minute), and

roughly included the polygon defined by the following Zone 13 UTM coordinates: (1) 238400 E, 4262600 N; (2) 232400 E, 4256700 N; (3) 235000 E, 4253600 N; and (4) 239500 E, 4258200 N.

In late April and May, prior to fawning, deer from the winter range experimental units migrated to summer range. We defined the summer range study area by movements of the radio-collared deer captured on winter range; summer range encompassed >1000 mi^2 covering the southern portion of the Uncompahgre Plateau and adjacent San Juan Mountains (Figure 2). The summer range study area extended north to the Dry Creek river drainage on the Uncompaghre Plateau, south to Mineral Creek near Silverton, CO, east to the Big Blue River drainage, and west to the San Miguel River canyon. However, a majority of the radio-collared deer summered on the Uncompahgre Plateau between Dry Creek to the north and Highway 62 to the south.

Winter range elevations ranged from 1830 m (6000 ft) in Shavano Valley to 2318 m (7600 ft) adjacent to the Dry Creek Rim above Shavano Valley. Winter range habitat was dominated by pinyon-juniper with interspersed sagebrush adjacent to agricultural fields in the Shavano and Uncompahgre Valleys. Summer range elevations occupied by deer ranged from 1891 m (6200 ft) in the Uncompahgre Valley to 3538 m (11,600 ft) in Imogene Basin southwest of Ouray, CO. Summer range habitats were dominated by spruce-subalpine fir (*Picea* spp.-*Abies lasiocarpa*), aspen (*Populus tremuloides*), sagebrush, ponderosa pine (*Pinus ponderosa*), Gambel oak (*Quercus gambelii*), and to a lesser extent, pinyon-juniper at lower elevations.

METHODS

Response Variables

We measured fetal and neonatal survival rates, early winter fawn:doe ratios, and overwinter fawn survival rates of deer occupying the treatment and control units. We delivered the nutrition enhancement treatment to deer from December through April, assessed fetus survival during June, measured neonate survival from June to December, and fawn:doe ratios during December–February (1 year after the treatment was initiated). We measured overwinter fawn survival from December to June in direct response to the current winter's treatment. Our measurements determined whether enhanced winter nutrition of adult does increased subsequent newborn fawn production and survival, and whether enhanced winter nutrition of 6–12-month old fawns increased overwinter fawn survival. Ultimately, these measurements provided an assessment of the effect of winter range habitat quality on yearling recruitment, and thus population productivity. We also measured overwinter and annual survival of adult does as a function of enhanced winter nutrition.

Sample Size

<u>Fetus/Neonate Survival</u>: Fetus and neonate sample sizes were not independent of one another because each resulted from the sample size of radio-collared does. We therefore needed a target sample size of either fetuses or neonates to generate our adult doe sample size. We based our sample size calculations on quantifying neonate survival because it was our highest priority and we could generate reliable estimates. Target fetus sample sizes were difficult to estimate because of uncertainty identifying fetus fates. That is, many fetuses measured *in utero* during winter were not accounted for as live or dead at parturition. Fetus survival rates could only be measured from some unpredictable fraction of the radio-collared doe sample, making sample size calculations of limited use. For neonate survival, a sample size of 40 neonates per experimental unit per year would provide power of 0.81 to detect a difference of 0.15 in survival between the 2 experimental units if survival among control fawns was 0.40. We assumed a control survival rate of 0.40 based on previous neonate survival rates measured on the Uncompahgre (Pojar and Bowden 2004) in combination with December fawn:doe ratios measured during the late 1980s and 1990s, when the Uncompahgre population declined (B. E. Watkins, Colorado Division of Wildlife,

unpublished data). We considered 40 neonates per experimental unit per year a minimum sample size because we ideally wanted to detect a difference in neonate survival of <0.15 between experimental units. Based on Bishop et al. (2002), we determined that 60 radio-collared does (30 treatment and 30 control) equipped with vaginal implant transmitters (VITs) would be necessary to capture a minimum of 80 newborn fawns. We also assumed that some fawns would be captured from other treatment and control radio-collared does not equipped with VITs. The 60 radio-collared does with VITs were also used to evaluate fetus survival; however, logistical constraints limited the power of fetus survival comparisons among experimental units.

<u>Early winter fawn:doe ratios</u>: We desired to detect an effect size, i.e., an increase in fawn:doe ratios in response to the treatments, in the range of 15 to 20 fawns per 100 does. These values were based on population models with overwinter fawn survival of 0.444, adult female survival of 0.853, and December fawn:doe ratios of 66 fawns per 100 does to obtain a stationary population (Unsworth et al. 1999). Based on surveys of the Uncompahgre deer population during the 1990s, the standard deviation of the fawns:100 does ratio for groups with at least one adult female was 57, with a mean of 41. Using an expected standard deviation of 57, the standard error of the mean fawns:100 does ratio for 40 radio-collared does is $57/(40^{1/2}) = 9.0$, which is the expected standard deviation of measured fawns:100 does ratios on each experimental unit. We assessed power using a two-sample *t*-test with a sample size of 4, representing the 4 years of the study where fawn:doe ratios were measured in response to enhanced nutrition. Our power to detect an increase of 20 fawns per 100 does based on classification of 40 radio-collared doe groups in each experimental unit was about 0.87.

<u>Overwinter fawn survival</u>: Our sample size of 40 fawns per experimental unit per year provided a power of 0.81 to detect a difference of 0.15 in survival between the 2 experimental units assuming a control survival rate of 0.40. We expected to see an increase in fawn survival (effect size) of approximately 0.15, because this was the difference measured in the density reduction experiment conducted by White and Bartmann (1998). We assumed a control survival rate of 0.40 based on long-term data from Colorado, Idaho, and Montana (Unsworth et al. 1999). However, recent data from 5 deer populations in Colorado indicates overwinter fawn survival has commonly been ≥70% during the past 6 years (Colorado Division of Wildlife, unpublished data).

Adult and 6-month Old Fawn Capture

We captured adult does and 6-month-old fawns during November and December using baited drop nets (Ramsey 1968, Schmidt et al. 1978) and helicopter net guns (Barrett et al. 1982, van Reenen 1982). We baited drop nets with certified weed-free alfalfa hay and apple pulp. We used drop nets as the principle capture technique for a 3–4 week capture period; we then used helicopter net-gunning at the end of the drop-net capture to secure the remainder of deer needed to meet our target sample sizes. All deer were hobbled and blind-folded after being captured. We used stretchers to carry deer away from the net when using drop nets. Deer were fitted with nylon-belting radio collars equipped with mortality sensors; pulse rate increased after remaining motionless for 4 hours. We placed permanent collars on adult females and temporary collars on fawns. To make collars temporary, we cut one end of the collar in half and reattached the two ends using rubber surgical tubing; fawns shed the collars ≥6 months post-capture. We stitched a rectangular piece of flexible plastic (Ritchey® neck band material) engraved with a unique identifier to the side of each collar. The unique identifier consisted of 2 symbols for adult females, and 1 symbol on 2 different colors of plastic for fawns. We used the identifiers to visually identify deer from the ground, which allowed us to effectively document use of the treatment, measure fawn:doe ratios, and assess experimental unit population size via mark-resight estimators. We recorded mass (kg), hind foot length (cm), and chest girth (cm) of each deer, and collected blood samples to evaluate disease prevalence.

During late February and early March, we captured an additional 30 adult female deer in each experimental unit by net-gunning. Captured deer were ferried by the helicopter to a central processing location, where deer were carried by stretchers to a tent for handling. We used ultrasonography to measure pregnancy status, fetal rate, and body condition of each captured deer. We retained and radio-collared pregnant does only. We then inserted a vaginal implant transmitter (VIT) in each doe as a technique for locating the timing and location of her birth site the following June. We also recorded the weight (kg), hind foot length (cm), and chest girth (cm) of each deer, and collected blood samples to evaluate disease prevalence.

Body Condition and Reproductive Status

We estimated body fat of treatment and control adult does during mid-late winter using an Aloka 210 (Aloka, Inc., Wallinford, Conn.) or SonoVet 2000 (Universal Medical Systems, Bedford Hills, NY) portable ultrasound unit with a 5 MHz linear transducer. We measured maximum subcutaneous fat thickness on the rump (MAXFAT) following the methodology of Stephenson et al. (1998, 2002). We also measured thickness of the longissimus dorsi muscle via ultrasound (Cook et al. 2001, Stephenson et al. 2002). A small area of hair was shaved to ensure contact between the transducer and the skin. Lubricant was applied to the shaved area for conduction purposes and fat and muscle thickness were measured using electronic calipers. We coupled the ultrasound measurements with body condition scores (BCS) obtained from palpation of the ribs, withers, and rump (Cook 2000). MAXFAT and rump BCS measurements were combined into a condition index used to estimate percent body fat (Cook and Cook 2002): % Fat = $-6.6387617 + 7.4271417x - 1.11579443x^2 + 0.07733803x^3$ where x = rLIVINDEX = (MAXFAT – 0.15) + rump BCS (if MAXFAT < 0.15, then rLIVINDEX = rump BCS). The rLIVINDEX and body fat regression was initially developed and validated for elk by Cook et al. (2001), and then modified by incorporating a validation of MAXFAT for mule deer performed by Stephenson et al. (2002).

We also evaluated differences in serum thyroid hormone concentrations between treatment and control adult does during mid-late winter. Specifically, we measured total thyroxine (T4), free T4 (FT4), total tri-iodothyronine (T3), and free T3 (FT3) following the methodologies of Watkins et al. (1983, 1991). Blood samples were collected at the time of capture, and serum hormone analyses were performed by the Michigan State University Animal Health Diagnostic Laboratory (East Lansing, Michigan). We compared serum thyroid hormone concentrations between treatment and control adult does, and also compared hormone levels to body fat estimates derived from the ultrasonography.

We quantified reproductive status (Stephenson et al. 1995, Andelt et al. 2004) with ultrasound via transabdominal scanning using a 3 MHz linear transducer. We searched for fetuses by scanning a portion of the abdomen that was shaved caudal to the last rib and left of the midline. We systematically searched each uterine horn to identify fetal numbers ranging from 0 to 3. Whenever possible, we measured eye diameter of each fetus to approximately estimate fetal age and parturition date.

Vaginal Implant Transmitters (VITs)

We used VITs manufactured by Advanced Telemetry Systems, Inc. (Isanti, MN). The VIT was 76 mm long, excluding antenna length, and had 2 silicone wings with a width of 57 mm when fully spread apart. The silicone wings were used to retain the transmitter in the vagina until parturition. The VIT weighed 15 grams and contained a 10–28 lithium battery programmed to a 12-hour on/off cycle. The diameter of the transmitter (excluding wings) was 14 mm, and was encased in an impermeable, water-proof, electrical resin. The transmitter contained an embedded heat-sensor which dictated the frequency pulse rate. When the heat sensor dropped below 90°F, synonymous with transmitter expulsion from the deer, the pulse rate changed from 40 PPM to 80 PPM. VIT batteries were programmed to be active from 0430 to 1630 hrs prior to daylight savings, and thus were active from 0530 to 1730 hrs after daylight savings and during the fawning period. We inserted VITs into deer using a vaginoscope

(Jorgensen Laboratories, Inc., Loveland, CO) and alligator forceps. The vaginoscope was 6” long with a 5/8” internal diameter and had a machined end (smooth surface) to minimize trauma when inserted into the vagina. A discreet mark was placed on the applicator showing approximate insertion distance. We obtained the length of a typical mule deer vaginal tract by taking measurements from road-killed deer and other fresh deer carcasses obtained in the study area.

Prior to use in the field, VITs were sterilized using chlorhexidine, air-dried, and sealed in a 3” × 8” sterilization pouch. We used sterilization containers with diluted chlorhexidine on site during capture to sterilize the vaginoscope and alligator forceps between each use. We used a new pair of nitrile surgical gloves to handle the vaginoscope and VIT for each deer. To insert a VIT, the plastic wings were folded together and placed into the end of the vaginoscope. We liberally applied sterile KY Jelly® to the scope and inserted it into the vaginal canal until the tip of the VIT antenna was approximately flush with the vulva. We used the alligator forceps, which extended through the vaginoscope, to firmly hold the VIT in place while the scope was pulled out from the vagina. The VIT silicone wings spread apart upon removal of the scope to hold the transmitter in place. The transmitter antenna was typically flush with the vulva, but on occasion extended up to 1 cm beyond the vulva. The tip of the antenna was encapsulated in a wax bead to protect the deer. All capture and handling procedures, including VIT techniques, were approved by the Colorado Division of Wildlife’s Animal Care and Use Committee (project protocols 11–2000 and 1–2002).

Neonate Fawn Capture

All radio-collared adult does were relocated from the air during late May to identify likely fawning areas. During each morning of June we checked VIT signal status by aerially relocating radio-collared does having VITs. Implant radio-signals could not be easily monitored from the ground because of weak signal strength and a large study area. Flights began at 0530 hours and were usually completed by 1000–1100 hours. Early flights were necessary to detect fast signals because temperature sensors of VITs expelled in open habitats and subject to sunlight often exceeded 90°F by mid-day, which caused VITs to switch back to a slow (i.e. prepartum) pulse. When a fast (i.e. postpartum) pulse rate was detected, we ground-tracked both the VIT and radio-collar frequencies simultaneously because the shed VIT and adult doe were typically in close proximity to one another. We attempted to observe behavior of the collared doe, establish whether the VIT was shed at a birth site, and search for fawns in the vicinity of the doe and expelled VIT. In cases where the doe had moved away from the VIT (e.g. >200 m), we located the VIT to determine whether shedding occurred at a birth site and whether any stillborn fawn(s) were present, and subsequently located the collared doe to search for fawns at her location. We attempted to account for each doe’s fetuses as live or stillborn fawns in order to quantify *in utero* fetus survival from February to birth. All personnel wore surgical gloves when handling fawns to help minimize human scent. We placed a drop-off radio-collar on each live fawn; radio collars were constructed with elastic neck-band material to facilitate expansion. Hole-punched, vinyl-belting tabs extended from the end of the elastic and from the transmitter for attachment purposes. We made collars temporary by cutting the vinyl tab extending from the elastic and reattaching the belting with latex tubing, which caused the collars to shed from the animal >6 months post-capture. Some collars were shed prematurely (i.e. 4–5 months post-capture) in association with fences during fall migration. For each fawn, mass (kg) and hind foot length (cm) were recorded, and a nasal swab sample was collected to screen for Bovine Viral Diarrhea. We then recorded basic vegetation characteristics of the birth site and promptly exited the site.

We ground-relocated most of the radio-collared does not receiving VITs approximately every other day during June in an attempt to capture additional fawns from treatment and control does. We did the same for any VIT doe whose implant failed because of premature expulsion or battery failure. We relied on doe behavior and searches in the vicinity of the collared does to locate fawns. We worked in pairs and partitioned the study area into segments, whereby each 2-person team was responsible for one segment. We used 3–4 teams during 2002 and 5–6 teams during 2003 and 2004.

Measurement of Survival Rates and Fawn:Doe Ratios

We measured survival rates by radio-monitoring collared deer from the ground and air to determine fate (i.e. lived or died). We also attempted to determine the cause of each mortality, with a primary goal of distinguishing between predation and non-predation mortality causes. We radio-monitored deer from the ground on a daily basis year-round and from the air on approximately a biweekly basis. We detected signals from nearly all radio-collared deer each day during winter, which typically allowed us to arrive at mortality sites within 24 hours of the mortality event. During summer and migration periods, deer were distributed widely and thus were more difficult to radio-monitor. All radio-collared neonates were checked daily throughout the summer and fall, whereas some adult and yearling deer could not be ground-monitored on a routine basis. In result, we typically located neonate mortalities within 24 hours of death, but some adult deer mortalities were not detected for several days, or on rare occasion, for one or more weeks. Fresh, intact neonate carcasses were collected and submitted to the Colorado Division of Wildlife's Wildlife Health Laboratory or the Colorado State University Diagnostic Laboratory for necropsy and tissue analyses. Fresh, intact adult and 6-month-old fawn carcasses were also submitted for laboratory necropsy when feasible. Field necropsies were performed on all other deer mortalities, and when appropriate, tissue samples were collected and submitted for analysis.

Each winter we used the radio-collared does to measure fawn:doe ratios in each experimental unit. The resulting fawn:doe ratio was a measurement of the previous year's treatment effect. We measured fawn:doe ratios using 2 techniques: (1) We located the sample of radio-collared does in each experimental unit from a fixed-wing airplane, and used the set of locations to define boundaries for the experimental unit. Shortly after (i.e. 1–2 days), we used a helicopter to systematically fly the defined unit and classify all deer groups encountered. For each group, we documented whether a radio-collared doe was present. (2) We located each radio-collared doe by radio telemetry from the ground. The group of deer with the collared doe was counted and classified by age and sex. Both methods were employed to gather as much information as possible to determine whether there was a treatment effect. The "true" value cannot be measured perfectly because of the inherent biases and potential sources of error associated with each technique. Thus, by employing both techniques, we had a greater chance of fully understanding whether the treatment caused an effect.

Treatment Delivery

We enhanced deer nutrition in the treatment experimental unit by providing a safe, pelleted supplemental feed. The supplemental feed was developed through extensive testing with both captive and wild deer (Baker and Hobbs 1985, Baker et al. 1998), and has been safely used in both applied research and management projects. We distributed pellets daily using 4wd pickup trucks, ATVs, and snowmobiles on primitive roads throughout the experimental unit to provide a food source for the entire deer population in the treatment unit. We carried each 50 lb. bag of pellets ≤200 m from the vehicle and distributed it by hand in approximately 20–30 small piles of feed in a linear fashion. We distributed numerous bags in successive order to create straight lines of feed that spanned most of the treatment area, which prevented animal concentrations. Our feeding technique also prevented dominant animals from restricting access to the food supply because of the large area over which pellets were distributed. We attempted to supply pellets ad libitum such that residual pellets remained when the next day's ration was provided. We closely monitored collared deer to ensure that treatment deer remained in the experimental unit and actually consumed the feed, and to make sure that non-treatment deer remained in the control unit, which they did. The few treatment adult does that distinctly moved away from the treatment unit were withdrawn from the sample for purposes of measuring treatment effects. However, to avoid any biases, all 6-month-old fawns captured in the treatment unit were included in survival analyses regardless of whether they accessed the supplement or not. Some fawns died shortly after capture (i.e. 2–3 weeks), before we could document whether they had access to the feed. Censoring these individuals would have biased treatment survival high relative to control survival. Also, very few fawns that survived more than 2–3 weeks moved away from the treatment unit.

The pelleted ration was commercially produced in the form of 2×1×0.5-cm wafers (Baker and Hobbs 1985). Feed quality (e.g. digestible energy, protein) vastly exceeded those of typical winter range deer diets; exact constituent values are provided by Baker et al. (1998). When provided ad libitum, the feed should have allowed deer to meet or exceed nutritional requirements for growth and maintenance (Ullrey et al. 1967, Verme and Ullrey 1972, Thompson et al. 1973, Smith et al. 1975, Baker et al. 1979, Holter et al. 1979). The basis for feeding such high quality pellets was to ensure that the treatment (enhanced nutrition) was effectively delivered to the deer. Our intent was not to determine the exact level of nutrition necessary to increase fawn recruitment, but rather to determine if nutrition was a significant limiting factor to recruitment. We will rely on habitat manipulation treatments to evaluate what exactly can be done via management to increase fawn survival and recruitment if nutrition is deemed a critical limiting factor.

Statistical Methods

We estimated deer numbers in each experimental unit during the first year of research using helicopter and ground mark-resight surveys. We used the joint hypergeometric maximum likelihood estimator for helicopter surveys and the Bowden estimator for ground surveys, and we analyzed data in Program NOREMARK (Neal et al. 1993, Bowden 1993, White 1996). We used a general linear model in PROC GLM in SAS (SAS Institute 1989) to test for differences in estimated percent body fat between treatment and control adult does and a multivariate model to test for differences in T4, FT4, T3, and FT3 thryoid hormones between treatment and control does. We used PROG REG (SAS Institute 1989) to evaluate the relationship between estimated percent body fat and serum thyroid hormone concentrations. We entered all fawn:doe ratios from helicopter surveys into the CDOW Deer, Elk, and Antelope Management (DEAMAN) database (G. C. White, Colorado State University, software) and computed standard errors based on groups (Bowden et al. 1984). We analyzed fawn:doe ratios from ground surveys using PROC MIXED in SAS (SAS Institute 1997). We used a reduced model with experimental unit as the independent variable; we considered experimental unit as a fixed effect and radio-collared does within an experimental unit as random effects. We analyzed fetus survival with a binomial survival rate from the subset of does where all fetuses had known fates. We also indirectly analyzed fetus survival by comparing the February fetus rate with the number of live newborn fawns/doe observed in June using a change-in-ratio estimator (White et al. 1996). We estimated neonate and overwinter fawn survival and adult doe survival using a Kaplan-Meier survival analysis (Kaplan and Meier 1958, Pollock et al. 1989), and we contrasted survival among experimental units using chi-square analyses. We used a common entry date for analyzing neonate survival because staggered entry would have biased survival rates low due to early mortalities that occurred before most of the sample was captured. We analyzed continuous fetus-neonate-overwinter fawn survival from March of one year to June of the following year using a staggered-entry Kaplan-Meier survival analysis (Pollock et al. 1989). All neonates were entered into the survival analysis on a common date rather than the exact date of capture for the same reason mentioned above. We computed the finite rate of increase, λ, for treatment and control deer by constructing a deterministic age-structured population model using measured pregnancy and fetus rates, fetus survival, neonate survival, overwinter fawn survival, and annual adult doe survival. Results are based on preliminary analyses and should be treated as such. Other results are presented as data summaries incorporating means and standard errors, or in some cases, raw data values.

RESULTS AND DISCUSSION

Deer Capture

During November and December 2000–2003, we captured and radio-collared 139 adult female mule deer evenly distributed among the treatment and control units. We also captured and radio-collared 241 6-month-old fawns during November and December 2001–2003 (40 fawns/unit/year). Due to budgeting constraints, we were unable to radio-collar 6-month old fawns during 2000. We captured an additional 154 adult females during late February and early March 2002–2004 and equipped them with radio collars and VITs. During June 2002–2004, we captured and radio-collared 276 newborn fawns from radio-collared adult females. Thus, the following results are based upon radio-monitoring of 810 individual mule deer evenly distributed among treatment and control units during November 2000–June 2004.

Treatment Delivery

2000–01: We distributed 88 tons of supplemental pellets from December 15, 2000, through April 19, 2001. We distributed an average of 0.85 tons of feed each day throughout 22 feeding sites across the 2.3 mi^2 treatment unit during most of the winter and spring. Deer were fed ad libitum because there was always residual feed remaining the next day during the feeding routine. We distributed each sack in approximately 20–30 distinct, small piles, resulting in >1000 small piles of feed throughout the treatment unit. Deer were able to effectively access the feed in small groups, and no aggression was ever observed among deer seeking access to the feed. Deer adapted to the pelleted supplement immediately and utilized it extensively throughout the winter. We continually monitored deer use of the feed from ground observation points, where we obtained 440 visual observations of radio-collared does consuming the feed. These observations, coupled with daily radio-monitoring and periodic aerial relocations, indicated 32 of the 37 radio-collared treatment does spent the entire winter and spring within the boundaries of the treatment unit and received the supplement on a daily basis.

Mark-resight population estimates from March helicopter (489 deer, SE = 62) and ground (494 deer, SE = 81) surveys, coupled with feed consumption, indicated we fed roughly 450 to 500 deer during most of the winter and spring. Feed consumption declined coincident with spring green-up, although deer continued to use the feed through mid-late April, at which point they began migrating to summer range. We also fed approximately 25 to 30 elk, but the elk did not affect deer access to the feed. Deer in the control experimental unit did not receive feed or any other treatment. Based on helicopter mark-resight surveys, the deer density in the treatment unit in December was 120 deer/mi^2 (SE = 9), but increased shortly after and was 213 deer/mi^2 (SE = 27) in March. Deer densities in the control unit changed little from 83 deer/mi^2 (SE = 12) in December to 101 deer/mi^2 (SE = 14) in March.

2001–02: We distributed 194 tons of the supplement throughout the treatment unit from December 15, 2001, through April 25, 2002. We distributed 2.0–2.1 tons of feed each day for most of the winter and spring. The large increase in supplement distribution from the previous year occurred because a large number of elk descended into the Uncompahgre Valley during late fall. Elk arrived in unusually large numbers throughout much of the valley prior to the onset of treatment delivery. Once feeding was initiated, approximately 300–500 elk adapted to the feed and remained in or around the treatment unit throughout most of the winter.

We could not deliver >2.1 tons of pellets per day given myriad logistical and budgetary constraints. Feed was not delivered ad libitum to all deer and elk in the treatment unit throughout the winter because residual feed was rarely observed during the next day's distribution. However, daily field observations indicated most deer approached ad libitum consumption of the supplement. In contrast to the previous winter, deer were waiting for the daily supplement to arrive each morning. Deer then consumed the supplement immediately after it was distributed. Elk were rarely observed utilizing the feed until late morning or afternoon, and elk continued to forage in fields below the treatment unit, whereas deer did not. We observed numerous radio-collared deer consuming pellets each day; not all of these observations were recorded because of time constraints with distributing the feed. Given this time limitation, we still recorded 818 observations of radio-collared deer consuming the supplemental feed (497 collared doe observations and 321 collared fawn observations). We observed 100–300 deer utilizing the pellets most days during the course of distributing the supplement. These observations rarely included elk; thus, direct deer-elk competition was minimized because of temporal differences in feeding, and deer had first access to the feed.

2002–03: We switched the treatment and control units consistent with the cross-over experimental design in December 2002. We distributed 97 tons of supplement from December 15, 2002 through April 30, 2003 across the new treatment unit, which had been the control unit the previous 2 years. The supplement was distributed daily throughout 29 sites over a larger area (~7 mi^2) than the first 2 years of research because of the greater size of the experimental unit and broader distribution of radio-collared deer. Residual feed was always present throughout the winter, thus deer were fed ad libitum. Only small groups of elk periodically accessed the supplement, and did not affect deer access. We obtained 286 observations of radio-collared deer consuming the supplement, which were difficult to obtain because the supplement was spread out over a large area and only a single feed site could be observed at any given moment. We also used daily ground radio-monitoring and periodic aerial relocations to document deer access to the supplement.

2003–04: We distributed 197 tons of pellets throughout the treatment unit from December 10, 2003, through April 30, 2004. The increase in supplement distribution occurred because elk numbers increased on the upper portion of the experimental unit. However, unlike winter 2001–02, residual feed was present throughout the winter and deer were fed ad libitum. We restricted elk to the upper extent of the deer winter range for most of the winter by allocating a portion of the daily feed distribution exclusively to elk. Thus, elk had a minimal affect on deer access to the supplement. We obtained 413 observations of radio-collared deer consuming the supplement. As before, we also used daily ground radio-monitoring and periodic aerial relocations to document deer access to the supplement.

Body Condition

Estimated percent body fat of adult does during late February and early March, 2002–2004, was higher for treatment deer than control deer ($F_{1, 148} = 153.41$, $P < 0.001$). Over all years combined, mean predicted body fat was 9.8% (SE = 0.36) for treatment adult does and 4.3% (SE = 0.26) for control does. The interaction of experimental unit × year for predicted body fat was also significant ($F_{2, 148} = 14.39$, $P <$ 0.001). This interaction occurred because the difference in body fat between treatment and control deer was greater during 2003 than during 2002 or 2004. Mean predicted body fat was 8.2% (SE = 0.92) for treatment adult does and 5.0% (SE = 0.71) for control does during 2002, and 9.0% (SE = 0.53) for treatment does and 4.7% (SE = 0.36) for control does during 2004. The difference was greater during 2003, where mean predicted body fat was 11.7% (SE = 0.35) for treatment does and 3.4% (SE = 0.35) for control does. The body fat estimates reported here should accurately reflect deer, but may be further refined in the future as additional research provides more data on the relationship between body condition indices and estimated percent body fat.

Serum thyroid hormone concentrations, measured during 2003 and 2004, were higher in treatment does than control does ($F_{4, 108} = 46.59$, $P < 0.001$) (Table 1). Hormone concentrations also varied between years ($F_{4, 108} = 14.21$, $P < 0.001$), but the experimental unit × year interaction was not significant ($F_{4, 108} = 1.68$, $P = 0.160$). Thus, each year thyroid hormone concentrations were higher in treatment does than control does. T4 was the most important thyroid hormone in describing the canonical variable for differences between treatment and control does (1.04*T4 – 0.02*T3 + 0.77*FT4 –

0.17*FT3). As expected, there was a high partial correlation between T4 and FT4 ($r = 0.67$, $P < 0.001$) and between T3 and FT3 ($r = 0.60$, $P < 0.001$), which has been documented previously (Watkins et al. 1983). When treated as 4 separate ANOVAs, T4 ($F_{1, 111} = 165.97$, $P < 0.001$), FT4 ($F_{1, 111} = 144.37$, $P < 0.001$), T3 ($F_{1, 111} = 13.84$, $P < 0.001$), and FT3 ($F_{1, 111} = 8.26$, $P = 0.005$) were significantly higher in treatment does than control does. Given these results, we evaluated the relationship between T4 concentrations and estimated percent body fat (derived from ultrasound and BCS indices) using a simple linear regression model (% Fat = –3.122 + 0.090*T4, $r^2 = 0.52$, $P < 0.001$). Similar correlations between T4 and actual percent body fat during mid-late winter have been previously documented for white-tailed deer and elk (Watkins et al. 1991, Cook et al. 2001).

Pregnancy and Fetus Rates

2002: Adult doe pregnancy rate was 0.95 (SE = 0.037, $n = 38$) in February–March 2002. We measured an average of 1.80 fetuses/doe (SE = 0.10, $n = 36$), which included 1.77 fetuses/doe (SE = 0.14, $n = 18$) in the treatment unit and 1.83 fetuses/doe (SE = 0.15, $n = 18$) in the control unit.

2003: Adult doe pregnancy rate was 0.92 (SE = 0.034, $n = 63$) in February–March 2003. Critical personnel and equipment for measuring fetus rates were not continuously available due to capture delays associated with helicopter mechanical problems. Some deer fetus counts were performed by inexperienced observers without optimum ultrasound equipment. VITs worked very well, though, allowing us to determine fetus numbers at parturition for many of the deer. Thus, we determined winter fetus rates by using the greatest fetus count for each individual deer, whether obtained using ultrasound during February–March or by locating newborn fawns and stillborns at birth sites during June. We were unable to determine a fetus count for 8 treatment deer because only pregnancy was established with ultrasound and no birth site assessments were possible in June. These 8 deer were removed from the fetus rate estimates. Of the 50 deer where a fetus count was obtained, 5 were yearlings (2 treatment yearlings, 3 control yearlings). We measured 1.74 fetuses/doe (SE = 0.069, $n = 50$) overall including yearlings, and 1.82 fetuses/doe (SE = 0.066, $n = 45$) excluding yearlings. Fetus rates with yearlings included were 1.77 fetuses/doe (SE = 0.091, $n = 22$) in the treatment unit and 1.70 fetuses/doe (SE = 0.10, $n = 28$) in the control unit.

2004: In February 2004, adult doe pregnancy rate was 0.94 (SE = 0.029, $n = 66$) and the fetus rate was 1.97 fetuses/doe (SE = 0.053, $n = 60$), which included 4 yearlings. Excluding yearlings, the fetus rate was 2.00 fetuses/doe (SE = 0.051, $n = 56$). Fetus rates were 1.90 fetuses/doe (SE = 0.074, $n = 30$) in the treatment unit and 2.03 fetuses/doe (SE = 0.076, $n = 30$) in the control unit with yearlings included, and 1.93 (SE = 0.069, $n = 29$) in the treatment unit and 2.07 (SE = 0.074, $n = 27$) in the control unit with yearlings excluded.

Pregnancy and fetus rates during our study equaled or exceeded other measured rates recorded in Colorado (Andelt et al. 2004), indicating moderate to high innate productivity potential for both treatment and control does. Our data also indicate that adequate numbers of bucks were available to breed does during the years of our study.

Fetus and Neonate Survival/Fawn:Doe Ratios

2000: Fawn:doe ratios were similar in the 2 experimental units in December 2000, prior to the first year's treatment delivery. Pre-treatment fawn:doe ratios were 52.6 fawns:100 does (SE = 5.3) in the Colona experimental unit and 51.6 fawns:100 does (SE = 5.0) in the Shavano experimental unit.

2001: We conducted 2 age classification helicopter surveys in the treatment and control units in late December 2001 and early January 2002, following the first year's treatment. On 23 December 2001, we observed 52.8 fawns:100 does (SE = 6.7) in the treatment unit and 36.7 fawns:100 does (SE = 3.8) in the control unit. On 8 January 2002, we observed 54.7 fawns:100 does (SE = 6.6) in the treatment unit and 50.5 fawns:100 does (SE = 6.0) in the control unit. During December 2001 – February 2002, we obtained fawn:doe ratio estimates from ground observations of radio-collared deer groups for both treatment and control deer. This survey resulted in 61.2 fawns:100 does (SE = 7.8) in the treatment unit and 74.5 fawns:100 does (SE = 8.5) in the control unit, although the result was not statistically significant ($t_{74} = 1.16$, $P = 0.249$). Our fawn:doe ratio results were conflicting and did not provide evidence that there was any treatment effect. We could not make any sound conclusions based on the data, although we generally concluded the nutrition enhancement treatment did not cause a substantial increase in neonatal production and survival during 2001. These data provided the incentive to incorporate direct measurements of fetus and neonate survival into our research.

2002: We measured fetus and neonate survival directly during March – December, 2002, following the second year's treatment; however, sample sizes were based on a technique assessment of VITs and were relatively small for contrasting survival rates among treatment and control fetuses and neonates (Bishop et al. 2002). During June 2002, we determined the fate of all fetuses (live or stillborn) from only 14 of 36 VIT does, largely because of a high VIT battery failure rate. Numbers of stillborns were similar among treatment and control deer, so we did not differentiate by experimental unit. The survival rate of fetuses ($n = 22$) from the 14 does was 0.86 (SE = 0.073). We also assessed fetus survival using a change-in-ratio estimator between the fetal rate measured in February–March and the observed number of live fawns/doe postpartum in June. In June 2002, considering all does ($n = 43$) that we located any fawn from, whether live or stillborn, we observed 1.42 (SE = 0.11) live fawns/doe postpartum. This rate should represent a conservative estimate of live fawns/doe postpartum because we inevitably failed to locate all live fawns from each doe. In other words, this estimate would treat any unaccounted fetuses (from the February measurement) as if they were stillborns. For radio-collared does that did not have VITs, and thus we did not have a winter fetus rate measurement, singletons would infer that either the deer only had 1 fetus, or that the other fetus died. It is likely that some of these singletons had a twin that we did not locate. This equates to a conservative fetus survival rate estimate of 0.79 (SE = 0.18).

Treatment fawn survival (Jun – Dec) was 0.613 (SE = 0.115, $n = 29$) and control fawn survival was 0.511 (SE = 0.108, $n = 25$). In late December 2002 and early January 2003, we once again conducted 2 age classification helicopter surveys in the treatment and control units. On 31 December 2002, we observed 91.9 fawns:100 does (SE = 8.4) in the treatment unit and 52.2 fawns:100 does (SE = 6.9) in the control unit. On 21 January 2003, we observed 52.6 fawns:100 does (SE = 6.4) in the treatment unit and 36.8 fawns:100 does (SE = 3.9) in the control unit. The combined helicopter survey data indicated 68.1 fawns:100 does (SE = 5.6) in the treatment unit and 42.8 fawns:100 does (SE = 3.5) in the control unit. Conversely, fawn:doe ratio estimates from ground classifications of doe groups during December 2002 – February 2003 were 47.7 fawns:100 does (SE = 6.3) in the treatment unit and 63.4 fawns:100 does (SE = 7.5) in the control unit ($t_{108} = 1.61$, $P = 0.110$). As in 2001, fawn:doe ratio results were conflicting. Helicopter survey data varied between 2 different flights, but consistently indicated a treatment effect. Ground classification data did not indicate a treatment effect.

2003: During June 2003, we determined the fate of all fetuses (live or stillborn) from 33 of 58 VIT does; we had better success because VITs commonly shed at birth sites. The survival rate of fetuses ($n = 58$) from these 33 does was 0.97 (SE = 0.024). In June 2003, incorporating all does ($n = 71$) from which we located any fawn, whether live or stillborn, we observed 1.49 (SE = 0.072) live fawns/doe postpartum. Using the change-in-ratio estimator described above, this results in an overall conservative fetus survival rate estimate of 0.86 (SE = 0.15). As in 2002, fetus survival was similar among treatment and control deer and not analyzed separately.

During June 2003, we captured and radio-collared 103 newborn fawns born from treatment and control radio-collared does (55 treatment fawns, 48 control fawns). The VITs worked well; we captured fawns from 41 of the 58 does fitted with VITs. Treatment fawn survival (Jun – Dec) was 0.624 (SE = 0.082) and control fawn survival was 0.483 (SE = 0.093). Final standard errors were larger than expected because a number of fawns shed collars prematurely when crossing fences during fall migration. Using helicopter surveys, we measured 62.4 fawns:100 does (SE = 5.3) in the treatment unit and 50.0 fawns:100 does (SE = 4.9) in the control unit. Estimates from ground classifications of doe groups were 68.0 fawns:100 does (SE = 7.6) in the treatment unit and 62.1 fawns:100 does (SE = 7.6) in the control unit. Age ratio estimates from the helicopter and the ground were more consistent during 2003 than in past years. Overall, observed fawn:doe ratios were consistent with treatment and control fawn survival rates measured from June to December.

<u>2004</u>: We determined the fate of all fetuses from 31 of 60 VIT does. The overall fetus survival rate was 0.90 (SE = 0.040, n = 58). Different from 2002 or 2003, all stillborns were from control does. The survival rate of control fetuses was 0.76 (SE = 0.085, n = 25) as compared to a survival rate of 1.00 (n = 33) for treatment fetuses. Using data from all does (n = 82) in which we located any fawn, the conservative change-in-ratio fetus survival estimate was 0.79 (SE = 0.13) overall, 0.88 (SE = 0.17) for treatment deer, and 0.69 (SE = 0.14) for control deer.

We captured and radio-collared 119 newborn fawns born from treatment and control radio-collared does during June 2004 (68 treatment fawns, 51 control fawns). Vaginal implants worked well again, and we had a large sample of non-VIT radio-collared does that we could relocate to opportunistically capture additional treatment and control fawns. Treatment fawn survival (Jun – Dec) was 0.438 (SE = 0.068) and control fawn survival was 0.414 (SE = 0.092). As in 2003, final standard errors were larger than expected because fawns shed collars prematurely during fall migration. Although neonate survival rates were similar among treatment and control fawns, fewer control fawns survived to December because of lower fetus survival. The proportion of fetuses measured in March that were born alive and survived to December during 2004 (i.e. fetus-neonate survival) was 0.438 (SE = 0.068) for treatments and 0.304 (0.073) for controls. Similar to 2002 and 2003, we observed higher December fawn recruitment among treatment deer based on measured survival rates. The difference during 2004 was that stillborn deaths factored in as a larger mortality factor among control deer than during 2002 or 2003. We measured 64.6 fawns:100 does (SE = 5.8) in the treatment unit and 52.7 fawns:100 does (SE = 5.1) in the control unit during helicopter surveys in 2004. Our ground classification estimates were 78.5 fawns:100 does (SE = 6.6) in the treatment unit and 68.7 fawns:100 does (SE = 5.1) in the control unit. Similar to 2003, observed fawn:doe ratios were consistent with treatment and control survival rates.

<u>*2002–2004 Fetus-Neonate Survival Summary*</u>: Fetus-neonate survival combined over all years of study (1 Mar–15 Dec, 2002–2004) was higher (χ^2_1 = 3.089, P = 0.079) for treatment deer ($S(t)$ = 0.519, SE = 0.048) than for control deer ($S(t)$ = 0.409, SE = 0.052). The high censor rate from shed collars during fall reduced power of the analysis and therefore increased standard errors and the resulting P-value. However, at roughly the same time neonate radio-collars were being shed, we captured new samples of fawns for measuring overwinter fawn survival. When fawns captured during November and early December were incorporated into the analysis via staggered entry, fetus-neonate treatment survival ($S(t)$ = 0.528, SE = 0.027) and control survival ($S(t)$ = 0.401, SE = 0.025) rates had tighter standard errors, which reduced the p-value associated with the survival rate comparison (χ^2_1 = 3.846, P = 0.050). The nutrition enhancement treatment had a positive effect on fetus and neonate survival through about the first month postpartum, at which point the treatment stopped having an effect (Figure 4). Fetus-neonate survival through 15 July, 2002–2004, was much higher (χ^2_1 = 6.013, P = 0.014) for treatment fawns ($S(t)$ = 0.746, SE = 0.035) than control fawns ($S(t)$ = 0.583, SE = 0.043). In summary, enhanced nutrition of adult does during winter and early spring caused higher survival of fetuses and fawns, resulting in higher December fawn recruitment (Figure 4).

2001–2004 Fawn:Doe Ratio Summary: Our results from 2001 and 2002 emphasize the inherent difficulties and biases associated with precisely measuring fawn:doe ratios, particularly in this research study. Ratios obtained from helicopter surveys were based on 2 short-duration flights/unit/year over spatially small units. Helicopter surveys were complicated by high deer densities in heavy cover, making both deer detection and fawn:doe classifications a considerable challenge. There were a variety of potential biases that may have affected the helicopter surveys, including differential sightability of does and fawns, double classification of some deer, and incorrect classification of yearling bucks with small antlers. Ground fawn:doe ratio observations of radio-collared doe groups were made using spotting scopes and field glasses, where we commonly studied the deer for some time. Incorrect classifications during these surveys were likely minimal. For example, small-antlered yearling bucks (e.g. 3 – 6" spikes) were detected from the ground, whereas they were undoubtedly missed on occasion during helicopter surveys. We also obtained repeated observations for some of the radio-collared doe groups from the ground. The main potential bias affecting ground fawn:doe classifications was how observations were made. Many of the ground classifications in the Shavano Valley experimental unit were made by radio-tracking does during the day. On the other hand, a majority of ground classifications in the Colona experimental unit were based on observing deer groups as they entered openings to feed during the late afternoon. Our age ratio results were more consistent with survival data during 2003 and 2004. Deer were not as concentrated during helicopter surveys, and unlike previous years, a majority of the ground classification data for the Colona experimental unit was obtained by radio-tracking does during the day rather than sitting and waiting for deer to emerge from pinyon-juniper hillsides to feed on sagebrush-grass benches.

We relied primarily on fetus-neonate survival data to make inferences regarding treatment effects because of the inherent difficulties measuring fawn:doe ratios in the 2 experimental units. However, we plan to compare observed helicopter and ground fawn:doe ratios with predicted ratios based on fetus-neonate survival data as a technique assessment of fawn:doe ratio measurements. This analysis will be incorporated into the job completion report.

Neonate Mortality Causes

2002–2003: During June – December of 2002 and 2003, 37 treatment fetuses-neonates died: 3 – stillborn, 8 – coyote predation, 2 – bear predation, 2 – felid predation, 3 – predation where the predator was undetermined, 11 – disease-starvation-malnutrition, 1 – abandonment, 3 – trauma-injury, 2 – unknown, and 2 – poached. The two poached fawns were censored from analyses evaluating the effect of the treatment. Converted to mortality rates based on the Kaplan-Meier survival analysis, 11.4% of all treatment fawns died from disease-starvation-malnutrition, 8.3% from coyote predation, 7.7% were stillborn, 3.1% died each from injury-trauma and from predation where the predator was undetermined, 2.1% each from bear predation, felid predation, and unknown causes, and 1.0% from abandonment. Simplified, 15.6% of all treatment fawns died from predation, 11.4% died from disease-starvation-malnutrition, 7.7% were stillborn, and 6.2% died from other or unknown causes. During June – December of 2002 and 2003, 38 control fetuses-neonates died: 2 – stillborn, 12 – coyote predation, 4 – felid predation, 2 – bear predation, 1 – predation where the predator was undetermined, 12 – disease-starvation-malnutrition, 1 – trauma-injury, and 4 – unknown. Converted to mortality rates based on the Kaplan-Meier survival analysis, 16.0% of all control fawns died from disease-starvation-malnutrition, 16.0% died from coyote predation, 5.3% each from felid predation and unknown causes, 4.9% were stillborn, 2.7% from bear predation, and 1.3% each from trauma-injury and predation where the predator was undetermined. Simplified, 25.3% of all control fawns died from predation, 16.0% from disease-starvation-malnutrition, 6.7% from other or unknown causes, and 4.9% were stillborn. In summary, mortality rates due to predation and disease-starvation-malnutrition were lower for treatment fawns than control fawns.

2004: During June – December, 2004, 36 treatment neonates died: 0 – stillborn, 13 – coyote or dog predation, 7 – bear predation, 3 – felid predation, 5 – predation where the predator was undetermined, 2 – disease-starvation-malnutrition, 1 – trauma-injury, and 5 – unknown. Converted to mortality rates based on the Kaplan-Meier survival analysis, 20.3% of all treatment fawns died from canid predation, 10.9% died from bear predation, 7.8% each from unknown causes and from predation where the predator was undetermined, 4.7% from felid predation, 3.1% from disease-starvation-malnutrition, and 1.6% from injury-trauma. Simplified, 43.7% of all treatment fawns died from predation, 9.4% died from other or unknown causes, and 3.1% died from disease-starvation-malnutrition. During June – December, 2004, 32 control fetuses-neonates died: 6 – stillborn, 5 – coyote predation, 4 – bear predation, 1 – felid predation, 2 – predation where the predator was undetermined, 4 – disease-starvation-malnutrition, 5 – injury-trauma, and 5 – unknown. We actually observed 9 stillborns from control does with fetus counts, although only 6 were associated with does in which all fetuses were accounted at parturition. Thus, we used only 6 of the stillborns in our estimate of fetus survival, and therefore stillborn mortality. Converted to mortality rates based on the Kaplan-Meier survival analysis, 24.0% of all control neonates were stillborn, 8.8% died each from coyote predation, injury-trauma, and unknown causes, 7.0% each from bear predation and disease-starvation-malnutrition, 3.5% from predation where the predator was undetermined, and 1.8% from felid predation. Simplified, 24.0% of all control fawns were stillborn, 21.0% died from predation, 17.5% died from other or unknown causes, and 7.0% died from disease-starvation-malnutrition.

Mortality causes were much different during 2004 than either 2002 or 2003. Predation rates were high on treatment fawns while stillborn mortality rates were high among control fawns. Several specific observations during 2004 are worthy of note. Three of the treatment fawn mortalities attributed to coyotes or dogs occurred amongst large herds of sheep which had been released to pasture immediately prior to the mortality events. Bear predation was higher among all fawns during 2004, although 3 of the 7 treatment bear mortalities involved triplets that were killed simultaneously by a bear 1–2 days after the fawns were born. Six treatment fawns captured in the same drainage tributary were killed within a 1-mi^2 area; the drainage was in a portion of the study area where no control fawns were captured. However, a single animal did not kill each of the fawns because the mortalities encompassed coyote, felid, and bear predation. Finally, we observed more accidental deaths than typical among control fawns. One control fawn drowned in a river, another fell, one became lodged in a water-filled mudhole, and both a control fawn and a treatment fawn died from injuries sustained while stuck in a woven wire fence.

2002–2004 Summary: Combining all years of data, the survival and cause-specific mortality rates of treatment fawns were: 52.8% survived, 27.2% died from predation (i.e. 13.3% canid, 5.7% bear, 3.2% felid, 5.1% undetermined), 8.2% died from disease-starvation-malnutrition, 4.2% were stillborn, and 7.6% died from other or unknown causes. Survival and cause-specific mortality rates of control fawns were: 40.1% survived, 24.3% died from predation (i.e. 12.9% canid, 4.6% bear, 3.8% felid, 3.0% undetermined), 12.1% died from disease-starvation-malnutrition, 12.1% were stillborn, and 11.4% died from other or unknown causes. The relatively high predation rate of treatment fawns was largely explained by 2004 data alone. As a general summary, control fawns suffered higher rates of disease, illness, malnutrition, and stillborn mortality (i.e. non-predator related mortalities) than did treatment fawns, which explains why survival was higher among treatment fawns (Figure 5).

Overwinter Fawn Survival and Mortality Causes

During winter 2001–02 (10 Dec 2001–15 Jun 2002), the survival rate of fawns was higher (χ^2_1 = 13.216, $P < 0.001$) in the treatment unit (S(t) = 0.865, SE = 0.056) than in the control unit (S(t) = 0.510, SE = 0.080). Similarly, in 2002–03 (10 Dec 2002–15 June 2003), the overwinter survival rate of fawns was higher (χ^2_1 = 5.734, P = 0.017) in the treatment unit (S(t) = 0.900, SE = 0.047) than in the control unit (S(t) = 0.691, SE = 0.074). Again in 2003–04 (10 Dec 2003–15 June 2004), the overwinter survival rate of fawns was higher (χ^2_1 = 3.852, P = 0.050) in the treatment unit (S(t) = 0.920, SE = 0.045) than in the control unit (S(t) = 0.756, SE = 0.067). Combining survival data across all 3 winters, treatment fawn survival (S(t) = 0.895, SE = 0.029) was 0.24 higher (χ^2_1 = 18.781, P < 0.001) than control fawn survival (S(t) = 0.655, SE = 0.044) (Figure 6). The treatment unit during winter 2001–02 became the control unit during winters 2002–03 and 2003–04, and vice versa. Thus, the overwinter survival treatment effect was replicated across each experimental unit. Fawn survival also varied as a function of early winter fawn mass (χ^2_1 = 21.19, P < 0.001). Surviving fawns averaged 3.5 kg heavier than fawns that died. The importance of early winter fawn mass as a predictor of overwinter survival has been documented previously (White et al. 1987, Bishop 1998, White and Bartmann 1998, Unsworth et al. 1999). Early winter mass of treatment fawns ($\bar{x}$ = 34.2 kg, SE = 0.418) was similar to control fawns ($\bar{x}$ = 34.4, SE = 0.423); thus the effect of the treatment was not confounded with pre-treatment fawn mass. It follows that fawns born from treatment does did not arrive to winter heavier than fawns born from control does, which was not necessarily surprising considering the treatment primarily effected neonate survival through about 1 month postpartum. In summary, the nutrition enhancement treatment improved overwinter fawn survival, and heavier fawns in each experimental unit had higher survival probabilities.

During winters 2001–04, 12 of 115 treatment fawns died: 5 from coyote predation, 3 from disease/illness, 2 from malnutrition, 1 from trauma-injury, and 1 unknown. Each of the 3 fawns that died from disease had adequate fat stores. At least one of these fawns died as a result of pneumonia. Converted to mortality rates based on the Kaplan-Meier survival analysis, 4.3% of all treatment fawns died from coyote predation, 2.6% from disease-illness, 1.7% from malnutrition, 0.9% from trauma-injury, and 0.9% from unknown causes. Simplified, 4.3% of all treatment fawns died from predation, 4.3% from disease-malnutrition, and 1.8% from other or unknown causes (Figure 7). During winters 2001–04, 41 of 120 control fawns died: 13 from coyote predation, 8 from mountain lion predation, 8 from malnutrition, 6 from unknown causes, 3 from predation where the predator was undetermined, 2 were road-killed, and 1 from trauma-injury. Converted to mortality rates based on the Kaplan-Meier survival analysis, 10.9% of all control fawns died from coyote predation, 6.7% from mountain lion predation, 6.7% from malnutrition, 5.0% from unknown causes, 2.5% from predation where the predator was undetermined, 1.7% from road-kill, and 0.8% from trauma-injury. Simplified, 20.1% of all control fawns died from predation, 6.7% from malnutrition, and 7.5% from other or unknown causes (Figure 7). Most fawns killed by predators had little or no femur marrow fat remaining, indicating the predation was likely compensatory in nature.

Fetus-Neonate-Overwinter Fawn Survival

We combined the preceding survival data into a single analysis to express the effect of the treatment across all stages of fawn production and survival. Using a staggered entry survival process with data combined over years, we estimated fawn survival from the fetus stage until one year of age, when fawns were recruited to the yearling (adult) age class (Figure 8). Survival of treatment fetuses to the yearling age class ($S(t)$ = 0.458, SE = 0.031) was 0.18 higher (χ^2_1 = 13.20, P < 0.001) than survival of control fetuses to the yearling age class ($S(t)$ = 0.276, SE = 0.026).

Adult Female Survival and Causes of Mortality

During winter 2000–01 (1 Dec 2000–31 May 2001), the adult doe survival rate in the treatment unit ($S(t)$ = 0.968, SE = 0.032) was greater (χ^2_1 = 2.649, P = 0.104) than the survival rate in the control unit ($S(t)$ = 0.861, SE = 0.058). However, annual adult doe survival rates (1 Dec 2000–30 Nov 2001) were similar among treatment and control deer (Trt: $S(t)$ = 0.839, SE = 0.066; Control: $S(t)$ = 0.833, SE = 0.062; χ^2_1 = 0.004, P = 0.947). We observed a similar result the following year. The 2001–02 overwinter adult doe survival rate in the treatment unit ($S(t)$ = 0.942, SE = 0.030) was greater (χ^2_1 = 3.116, P = 0.078) than survival in the control unit ($S(t)$ = 0.848, SE = 0.044), yet annual adult doe survival was similar among treatment and control deer (Trt: $S(t)$ = 0.824, SE = 0.049; Control: $S(t)$ = 0.818, SE =

0.047; χ^2_1 = 0.090, P = 0.764). Thus, mortalities of control deer occurred primarily during the winter months, while treatment does died primarily during the summer and fall months.

During winter 2002–03, following the treatment cross-over, overwinter adult doe survival rates were similar among treatment and control deer (Trt: $S(t)$ = 0.945, SE = 0.024; Control: $S(t)$ = 0.924, SE = 0.028; χ^2_1 = 0.360, P = 0.549). However, annual adult doe survival rates (1 Dec 2002–30 Nov 2003) were higher (χ^2_1 = 2.016, P = 0.156) for treatment does ($S(t)$ = 0.888, SE = 0.034) than control does ($S(t)$ = 0.813, SE = 0.041). The main difference from the previous 2 years was that overwinter survival of adult does in the Shavano experimental unit increased in 2002–03 upon receiving the treatment. Summer-fall survival was similar in that Colona adult does had higher mortality rates than Shavano adult does. Thus, in 2002–03, there was no difference between survival rates of treatment and control adult does during winter but there was evidence of higher annual survival of treatment adult does. During winter 2003–04, overwinter adult doe survival rates were higher (χ^2_1 = 3.843, P = 0.050) among treatment does ($S(t)$ = 0.979, SE = 0.014) than control does ($S(t)$ = 0.915, SE = 0.027). The annual adult doe survival rate (1 Dec 2003–30 Nov 2004) was 0.895 (SE = 0.030) for treatment does and 0.832 (SE = 0.036) for control does, which was marginally different (χ^2_1 = 1.562, P = 0.211). Considering all years, the treatment improved overwinter adult doe survival but had a relatively minor affect on annual survival. Considering only the past 2 years, the treatment had a positive affect on annual survival. Annual survival rates measured in this study align reasonably well with expected survival based on other studies (Unsworth et al. 1999, Bishop et al. 2005, B. E. Watkins, Colorado Division of Wildlife, unpublished data).

During 2000–02, when the Colona experimental unit received the treatment and the Shavano experimental unit was the control, 16 treatment and 16 control does died. The 16 treatment does died from the following categories: 4 – road-killed, 3 – while giving birth, 3 – predation (undetermined predator), 2 – non-predation unknown (intact carcasses with no evidence of predation or scavenging), 1 – disease (chronic arthritis), 1 – mountain lion predation, and 2 – unknown. Predation was not a major mortality factor for treatment does, and a majority of mortalities were independent of nutrition (does were in good condition). The 16 control doe mortalities included the following causes: 5 – mountain lion predation, 3 – malnutrition, 2 – non-predation unknown, 1 – road-killed, 1 – bear predation, 1 – fence injury, 1 – legal harvest, and 2 – unknown. Predation and malnutrition were the major mortality causes of control deer. Interestingly, during this 2-year period, we did not document any coyote predation on adult does.

During 2002–04, with Shavano as the treatment and Colona as the control, there were 20 treatment doe mortalities: 6 – disease/infection, 3 – coyote predation, 1 – road-killed, 1 – broken jaw which led to starvation, 1 – fence injury, 1 poached, and 7 unknown causes. As we saw during 2000-02, predation was not a major mortality factor for treatment does, and a majority of mortalities were independent of nutrition. We observed 33 control adult doe mortalities during the same time period: 8 – road-kill, 7 – malnutrition-disease, 5 – coyote predation, 3 – mountain lion predation, 3 – non-predation unknown, 1 – bear predation, 1 – predation where the predator was undetermined, and 5 – unknown causes. Road kill, malnutrition-disease, and predation were the major mortality factors of control does during 2002–04.

Road kill was a significant mortality factor of Colona adult does but not Shavano adult does, which partially explains why we failed to see a treatment effect during 2000–02 but did see one during 2002–04. If road-killed deer were censored, greater evidence would exist for a treatment effect during 2000–02 while there would be less evidence of a treatment effect during 2002–04. However, road-kill had minimal effect on the overall 4-year interpretation of the treatment effect on adult doe survival because of the cross-over design. Ignoring road kill, treatment does tended to die of causes unrelated to nutrition whereas control does were more susceptible to malnutrition and predation.

Population Growth Rate

The finite rate of population increase, λ, based on our measurements of treatment population parameters was 1.20 (Table 2), which would cause the deer population to double in approximately 4 years. The finite rate of increase calculated from control deer was 1.04 (Table 2), indicating a stable or slightly increasing population. The nutrition enhancement treatment therefore had a dramatic effect on deer population performance, indicating habitat quality was ultimately limiting the population.

SUMMARY

We successfully enhanced nutrition of deer occupying the treatment units based on our body fat estimates of treatment and control does. Pregnancy and fetus rates were similar among treatment and control does. The treatment caused an increase in both fetus-neonate survival and overwinter fawn survival, resulting in higher yearling recruitment. Overwinter adult doe survival increased as a result of the treatment, but annual survival was more similar among treatment and control adult does. Combining all parameter estimates into a deterministic population model, the treatment population indicated an exceptionally high rate of increase (λ = 1.20) while the control population (λ = 1.04) was indicative of the overall Uncompahgre deer population during 2000–2004. The nutrition enhancement treatment was artificial in the sense that we applied it only to test whether habitat quality was ultimately more limiting than predation or other factors. Our results to do not provide support for managing deer populations with nutrition supplements because our treatment delivery approach could not be applied to a large number of animals over a large area. Rather, our results provide a foundation for focusing deer management efforts on improving habitat quality in western Colorado pinyon-juniper ecosystems with corresponding research efforts to quantify the effects of habitat manipulations on deer.

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Table 1. Total thyroxine (T4) and total tri-iodothyronine (T3) concentrations (nmol/l), and free T4 (FT4) and free T3 (FT3) concentrations (pmol/l), measured during late February in adult female mule deer occupying a nutrition enhancement treatment unit and a control unit on the Uncompahgre Plateau in southwest Colorado, 2003–04.

		Thyroid Hormone			
Year	Exp. Unit	T4 (SE)	FT4 (SE)	T3 (SE)	FT3 (SE)
2003	Treatment	146.6 (3.53)	30.0 (1.27)	1.65 (0.058)	4.10 (0.130)
	Control	92.3 (3.56)	17.1 (0.65)	1.42 (0.080)	3.71 (0.210)
2004	Treatment	131.9 (4.48)	24.8 (1.39)	2.08 (0.075)	4.21 (0.154)
	Control	90.0 (3.54)	12.5 (0.59)	1.70 (0.104)	3.60 (0.188)

Table 2. Population parameter estimates and population finite rate of increase, λ, for treatment deer that received a nutrition enhancement and control deer that accessed existing habitat only, southwest Colorado, 2002–04.

Population Parameter	Treatment	Control
Adult doe pregnancy rate[a]	0.937	0.937
Adult doe fetus rate[a]	1.84	1.84
Fetus survival to birth	0.958	0.879
Neonate survival to December	0.551	0.456
Overwinter fawn survival to June	0.895	0.655
Annual adult doe survival	0.860	0.824
Finite Rate of Increase, λ	1.20	1.04

[a]We used overall estimates of pregnancy and fetus rates because we did not detect meaningful differences between treatment and control deer.

Year	Unit A	Unit B
2000-01	**Treatment**	Control
2001-02	**Treatment**	Control
2002-03	Control	**Treatment**
2003-04	Control	**Treatment**

Figure 1. Schematic representation of experimental units and nutrition enhancement treatment allocation. Units A and B were located in winter range habitat on the Uncompahgre Plateau in southwest Colorado. The nutrition enhancement cross-over design encompassed 4 years.

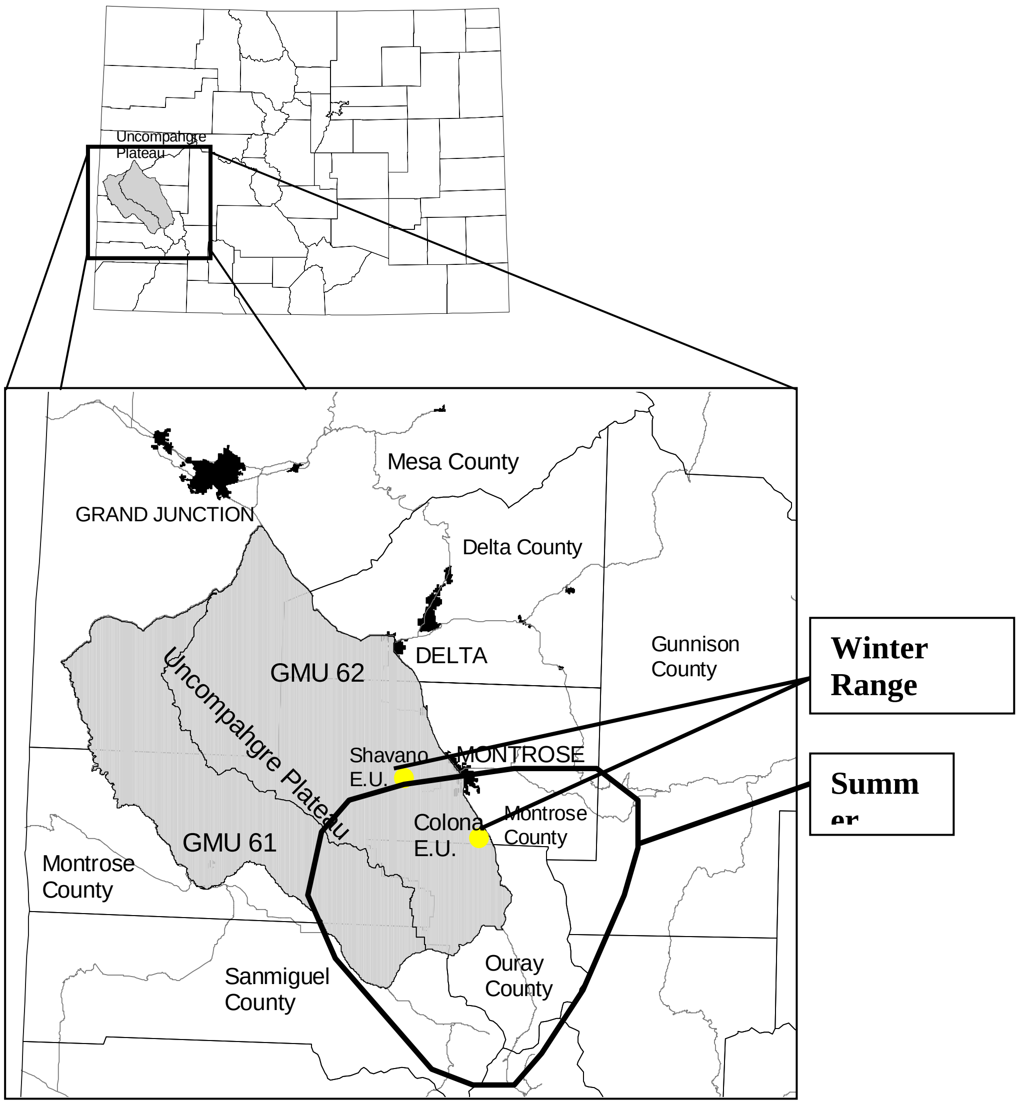

Figure 2. Location of Colona and Shavano (Units A and B) experimental units on the Uncompahgre Plateau, southwest Colorado; and location of the summer range study area encompassing the southern Uncompahgre Plateau and adjacent San Juan Mountains.

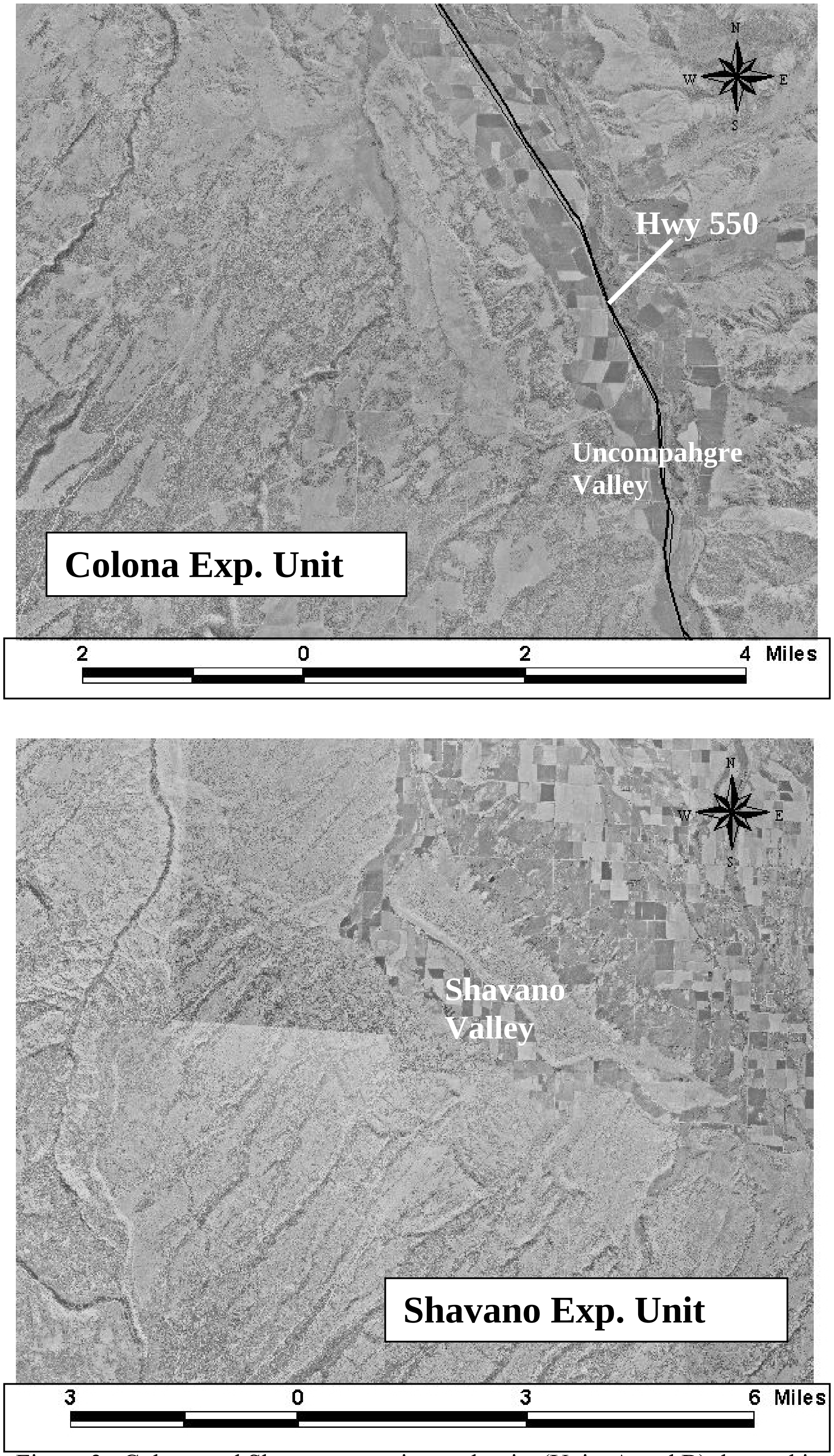

Figure 3. Colona and Shavano experimental units (Units A and B), located in Game Management Unit 62 on the Uncompahgre Plateau, southwest Colorado.

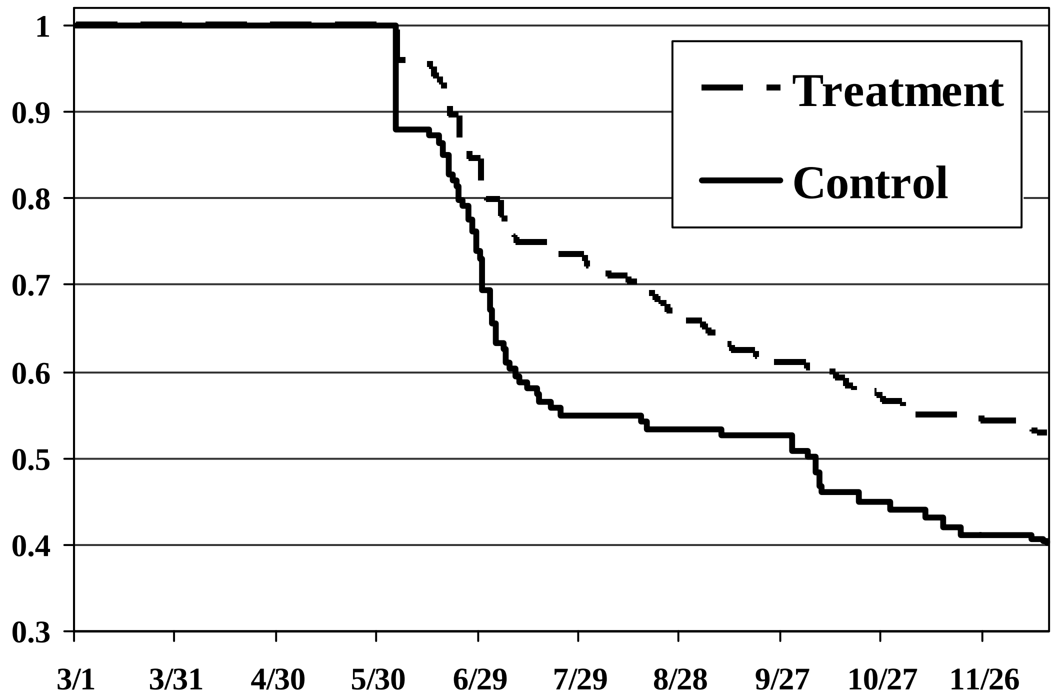

Figure 4. Survival (1 Mar –15 Dec, 2002–2004) of mule deer fetuses-neonates born from adult does receiving enhanced nutrition during winter (Treatment, S(t) = 0.528, SE = 0.027) and from adult does accessing existing winter habitat only (Control, S(t) = 0.401, SE = 0.025), southwest Colorado.

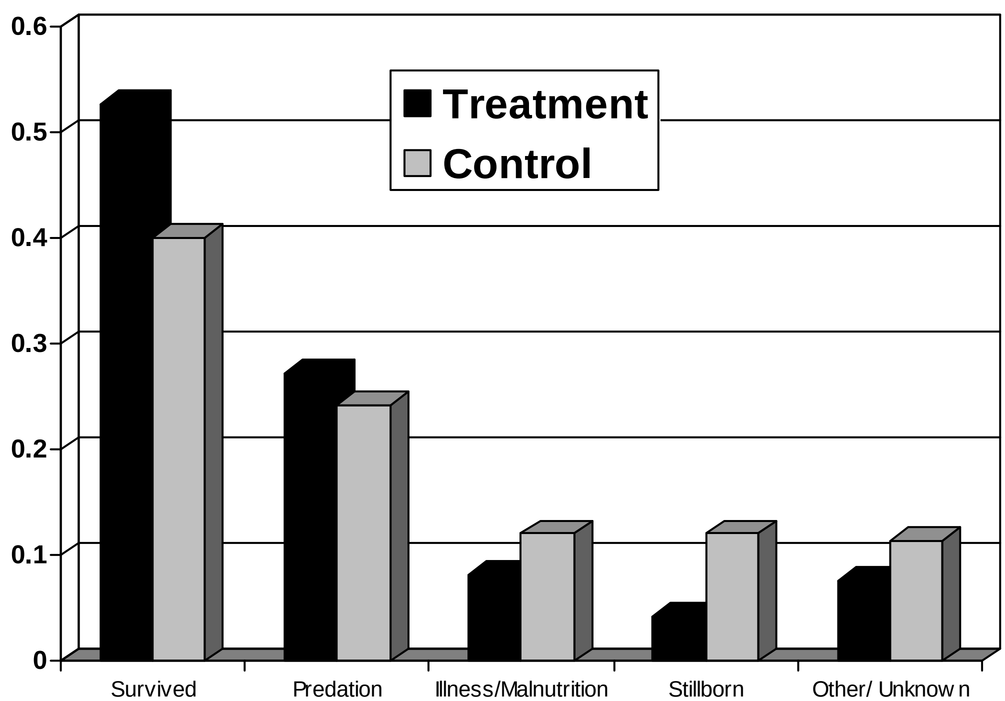

Figure 5. Survival and cause-specific mortality rates (1 Mar –15 Dec, 2002–2004) of mule deer fetuses-neonates born from adult does receiving enhanced nutrition during winter (Treatment) and from adult does accessing existing winter habitat only (Control), southwest Colorado.

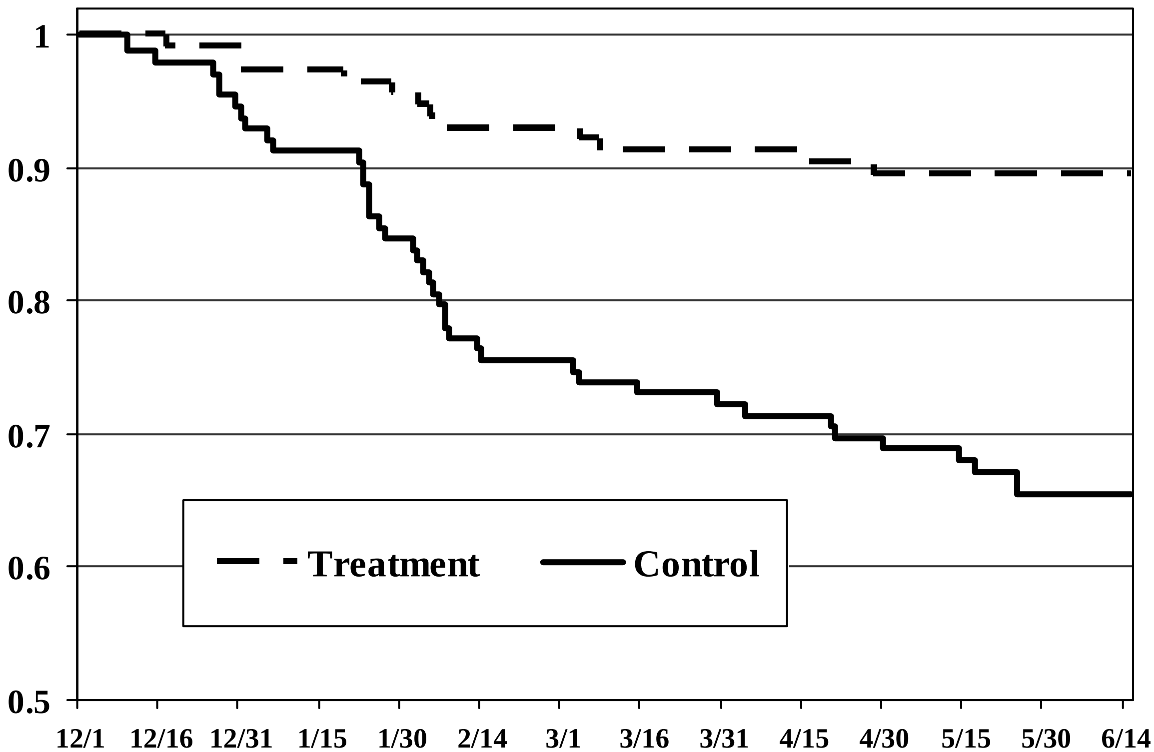

Figure 6. Overwinter fawn survival (10 Dec –15 Jun, 2001–2004) in a nutrition enhancement treatment unit ($S(t) = 0.895$, SE = 0.029) and a control unit ($S(t) = 0.655$, SE = 0.044) on the Uncompahgre Plateau, southwest Colorado.

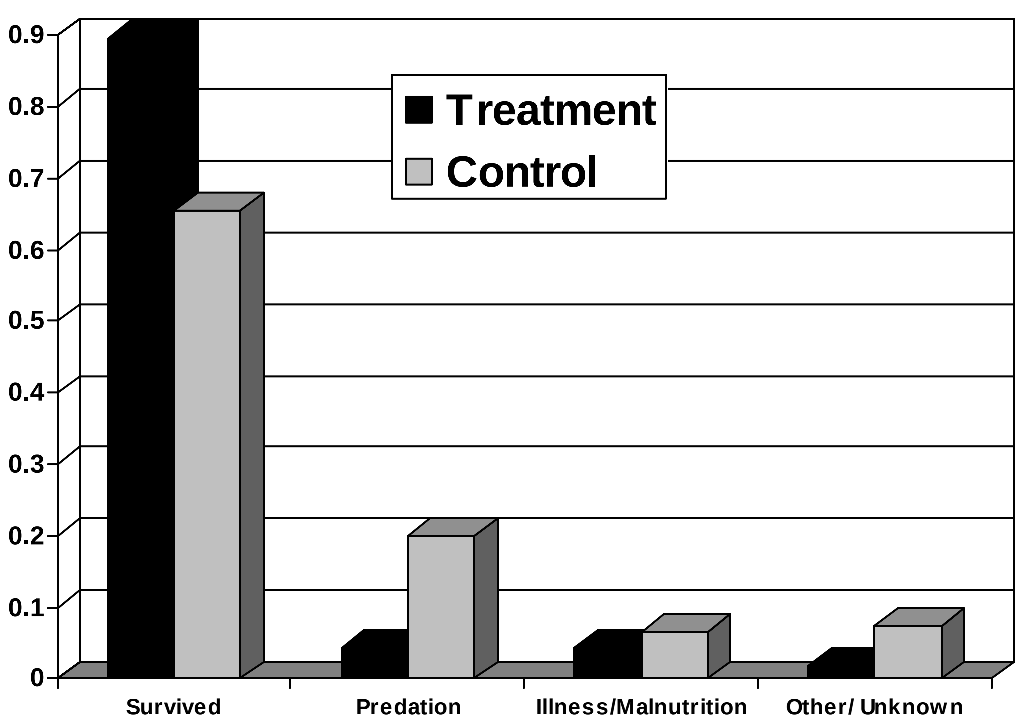

Figure 7. Overwinter fawn survival and cause-specific mortality rates (10 Dec–15 Jun, 2001–2004) in a nutrition enhancement treatment unit and a control unit on the Uncompahgre Plateau, southwest Colorado.

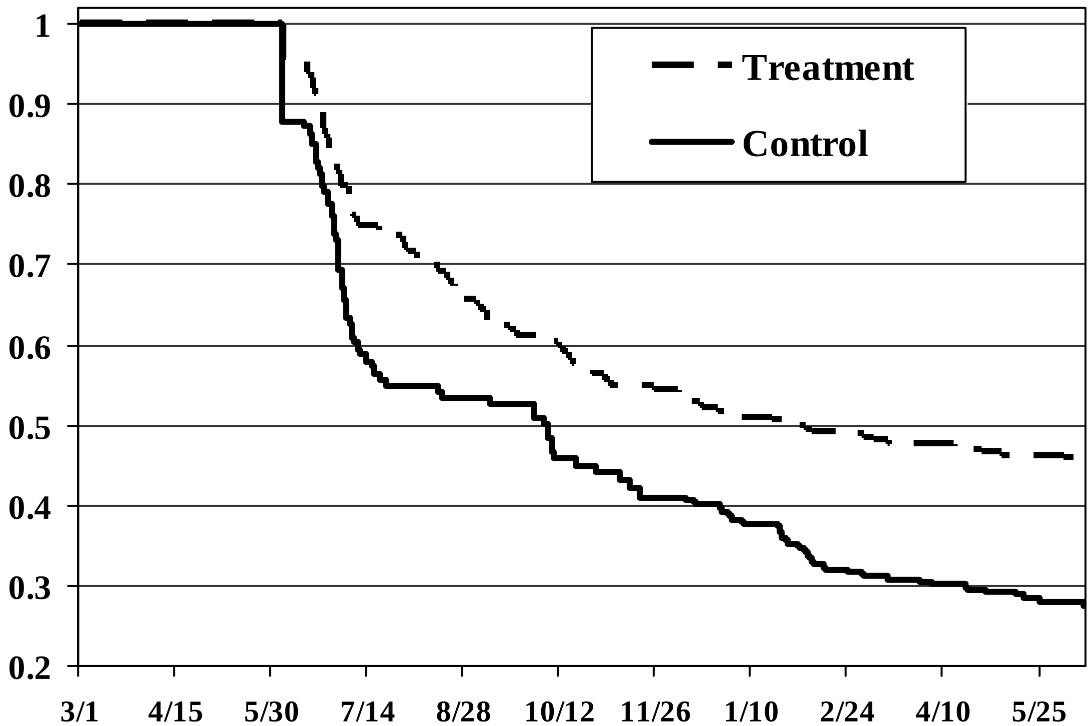

Figure 8. Fawn survival from fetus stage (March) to 1 year of age (June of the following year) for deer receiving enhanced nutrition during winter (Treatment, S(*t*) = 0.458, SE = 0.031) and deer accessing existing winter habitat only (Control, S(*t*) = 0.276, SE = 0.026), southwest Colorado, 2002–2004.

APPENDIX I

We submitted the following manuscript (referenced here by Abstract) to the Journal of Wildlife Management during summer 2005.

USING VAGINAL TRANSMITTERS TO CAPTURE NEONATES FROM MARKED MULE DEER

CHAD J. BISHOP, DAVID J. FREDDY, GARY C. WHITE, BRUCE E. WATKINS, THOMAS R. STEPHENSON, AND LISA L. WOLFE

ABSTRACT

Measuring reproductive success of previously-marked, adult female ungulates enables study of certain complex ecological factors limiting populations. We evaluated the effectiveness of using vaginal implant transmitters (VITs, *n* = 154) in mule deer (*Odocoileus hemionus*) combined with repeated relocations of other radio-collared deer for capturing effective samples of neonates (e.g. >100/year) from free-ranging, marked females. We also evaluated the effectiveness of VITs, when used in conjunction with *in utero* fetus counts, for obtaining direct estimates of fetus survival. During 2003 and 2004, when VIT batteries were placed on a 12-hour duty cycle to lower failure rates, the proportion of VITs that shed ≤3 days prepartum or during parturition was 0.623 (SE = 0.0456), and the proportion shed during parturition was 0.447 (SE = 0.0468). Our neonate capture success rate was 0.880 (SE = 0.0359) from does with VITs shed ≤3 days prepartum or during parturition and 0.307 (SE = 0.0235) from radio-collared does without VITs or whose implants failed to function properly. Combining techniques we captured 275 neonates and 21 stillborns during 2002–2004. We accounted for all fetuses at birth (i.e. live or stillborn) from 78 of the 147 does (0.531, SE = 0.0413) with winter fetus counts, which was heavily dependent on VIT retention success. Deer that shed VITs prepartum were larger and older than deer that retained implants to parturition, indicating a need to develop variable-sized VITs which may be individually fitted to deer in the field. We demonstrated that direct estimates of fetus and neonate survival may be obtained from previously-marked female mule deer in free-ranging populations, thus expanding opportunities for conducting field experiments. Resulting neonate survival estimates lacked bias that is typically associated with other neonate capture techniques. However, current vaginal implant failure rates and overall expense limit applicability of the technique to well-funded studies with adequate personnel.

Colorado Division of Wildlife
July 2004 – June 2005

WILDLIFE RESEARCH REPORT

State of Colorado	:	Division of Wildlife
Cost Center 3430	:	Mammals Research
Work Package 3001	:	Deer Conservaton
Task No. 5	:	Multispecies Investigations Consulting Services for Mark-Recapture Analysis
Federal Aid Project: W-185-R	:	

Period Covered: July 1, 2004 - June 30, 2005

Author: G. C. White

Personnel: C. Bishop, G. Miller, T. E. Remington, D. J. Freddy, T. M. Shenk, L. Stevens, J. Craig, R. Kahn, D. C. Bowden, F. Pusateri, J. Dennis, P. Schnurr, B. Andelt, A. Seglund, D. Finley, A. Linstrom, K. Strohm, P. Conn.

All information in this report is preliminary and subject to further evaluation. Information MAY NOT BE PUBLISHED OR QUOTED without permission of the author. Manipulation of these data beyond that contained in this report is discouraged.

ABSTRACT

Progress towards the objectives of this job include:

1. Consulting assistance to CDOW on harvest surveys, terrestrial inventory systems, and population modeling procedures was provided. Assistance with estimation of spring and fall turkey, spring snow goose, sharp-tailed and sage grouse, chukars, ptarmigan, Abert's squirrels, and general small game harvest was provided, and programs and harvest estimates provided to CDOW via email and CD ROM. Computer code written in SAS to compute these estimates and display results graphically was also provided. Computer code was also written in SAS to estimate the compliance rate of Colorado small game license holders with the Harvest Information Program.
2. The DEAMAN software package for the storage, summary, and analysis of big game population and harvest data was revised further as a Windows XP program. A User's Manual has been provided to terrestrial biologists via the WWW at http://www.cnr.colostate.edu/~gwhite/deaman. I met with the CDOW software group to discuss conversion of DEAMAN to a central server application.
3. Consultation with CDOW Terrestrial Biologists in the use of DEAMAN and population modeling procedures continued. Numerous questions were answered via meetings with biologists, and via email.
4. A paper on the estimation of mule deer population sizes in GMU 10 was published in the Wildlife Society Bulletin: Freddy, D. J., G. C. White, M. C. Kneeland, R. H. Kahn, J. W. Unsworth, W. J. deVergie, V. K. Grahm, J. H. Ellenberger, and C. H. Wagner. 2004. How many mule deer are there? Challenges of credibility in Colorado. Wildlife Society Bulletin 32:916-927.
5. A paper on the peregrine falcon population dynamics in Colorado was published in the Journal of Wildlife Management: Craig, G. R., G. C. White, and J. H. Enderson. 2004. Survival, recruitment, and rate of population change of the peregrine falcon population in Colorado. Journal of Wildlife Management 68:1032-1038.

6. A paper on the impact of limited antlered harvest on mule deer sex and age ratios was accepted for publication in the Wildlife Society Bulletin: Bishop, C. J., G. C. White, D. J. Freddy, and B. E. Watkins. 2005. Effect of limited antlered harvest on mule deer sex and age ratios. Wildlife Society Bulletin. In Press.
7. A paper on the estimation of the area of black-tailed prairie dog colonies in eastern Colorado was accepted for publication in the Wildlife Society Bulletin: White, G. C., J. R. Dennis, and F. M. Pusateri. 2005. Area of black-tailed prairie dog colonies in eastern Colorado. Wildlife Society Bulletin. In Press.
8. A paper on methodologies to obtain more rigorous population monitoring data was accepted for publication in Wildlife Research: White, G. C. 2004. Correcting counts: techniques to de-index. Wildlife Research. In Press.
9. A paper evaluating methods of estimating the impact of harvest on survival rates was published in Animal Diversity and Conservation: Otis, D. L., and G. C. White. 2004. Evaluation of ultrastructure and random effects band recovery models for estimating relationships between survival and harvest rates in exploited populations. Animal Biodiversity and Conservation 27.1: 157-173.
10. A paper on the procedures to monitor swift fox populations in eastern Colorado was accepted for publication in the Journal of Wildlife Management: Finley, D. J., G. C. White and J. P. Fitzgerald. 2004. Estimation of swift fox population size and occupancy rates in eastern Colorado. Journal of Wildlife Management. In Press.
11. A research study to examine the impact of nutrition on the decline of mule deer fecundity during the last 20 years was continued in cooperation with Chad Bishop. Portions of this work will serve as his doctoral dissertation.
12. A graduate research project (M. S.) to develop a sage grouse population model, using North Park sage grouse data to develop parameter estimates, was completed. The graduate student is Kristen Strohm and her thesis is "Sage Grouse Population Dynamics in North Park, Colorado".
13. A graduate research project (M. S.) To evaluate line transect methodology for estimating pronghorn populations in eastern Colorado was continued. The graduate student is Aaron Linstrom, and the project is in addition to his full-time duties as a terrestrial biologist with CDOW.
14. A graduate research project (Ph. D.) to develop statistical models to monitor puma and black bear populations in Colorado based on checks of harvested animals and DNA and/or radio-tracking data was continued (with funding for 04-05 through the CSU PRIMES program). The graduate student is Paul Conn.
15. Development of the design of a monitoring system for white-tailed prairie dogs in western Colorado and eastern Utah was continued. This effort is in cooperation with Pam Schnurr, Bill Andelt, and Amy Seglund.
16. Development of the design of a monitoring system for swift fox in eastern Colorado was continued, and data analysis for this project was initiated. This effort is in cooperation with Francie Pusatari and Darby Finley.

WILDLIFE RESEARCH REPORT

CONSULTING SERVICES FOR MARK-RECAPTURE ANALYSES

GARY C. WHITE

P. N. OBJECTIVE

Monitor swift fox populations in eastern Colorado.

SEGMENT OBJECTIVES

1. Extend a mark-recapture monitoring scheme to estimate occupancy rates of swift foxes (*Vulpes velox*) on 12-mi^2 quadrats in eastern Colorado.
2. Contrast estimates from the current survey with thoses obtained in 1998 and published in Finley et al. (2005).

ABSTRACT

A randomly selected sample of 15 ~12-mi^2 grids in eastern Colorado were trapped with a 4 × 5 grid of traps between August, 2004 and February, 2005. Swift foxes were trapped on 36 of the 51 grids, with 136 total fox captures. Comparison of the estimates of the percent of 12-mi^2 grids occupied by swift foxes in eastern Colorado does not appear to have changed since a comparable sample was taken of 72 grids in March, 1995 – January, 1997 (Finley et al. 2005). Using the average percentage of the grids in short grass prairie with the minimum AICc model, the earlier estimate was $\hat{\psi}$ = 0.821 (SE 0.0659), compared to the current estimate of $\hat{\psi}$ = 0.777 (SE = 0.0786). The estimated change is −0.044 (SE = 0.103, 95% CI −0.245 – 0.157). Summing the predicted occupancy values across the sampled grids for the respective studies provides a similar conclusion: Finley et al. (2005) found $\hat{\psi}$ = 0.790 (SE = 0.0574), whereas this study found $\hat{\psi}$ = 0.742 (SE = 0.0869), providing an estimate of the change of −0.048 (SE = 0.104, 95% CI −0.252 – 0.156). These differences are well within the sampling variation of the estimates, and do not suggest a decline in swift fox populations in eastern Colorado.

RESULTS

Sample of Grids

Finley et al. (2005) found that the covariate percent Short Grass Prairie (SGP) is a good predictor of the presence of foxes in eastern Colorado. The distribution of this covariate is bimodal (Figure 1). To build the best relationship between SGP and fox numbers, we sampled across this continuum of SGP values. Thus, the 2,566 trapping grids considered in the sampling frame of grids (Figure 1) to be trapped were sorted by the percentage of SGP predicted by the CDOW GIS system. Then a random start between 1 and 66 was picked, and every 50th grid was selected to be sampled. This procedure resulted in a sample of 51 blocks. When I multiply the frequency of the sample by 50, I obtain a close relationship between the sampling frame and the grids sampled (Figure 2).

Statistical Methods

Analysis methods to estimate occupancy rates followed the procedures of Finley et al. (2005), using the occupancy model of MacKenzie et al. (2002) in Program MARK (White and Burnham 1999). I considered a set of *a priori* models that incorporated month as sine and cosine functions to model detection probabilities (p), and the percentage of short grass prairie on the trapping grid to model both detection probabilities and probability of occupancy, ψ (psi). Model selection was performed with information-theoretic methods following Burnham and Anderson (2002).

Analysis methods to estimate the population of foxes using a trapping grid also followed Finley et al. (2005), using the Huggins estimator (Huggins 1989, 1991) to estimate population size. Model selection was performed with information-theoretic methods following Burnham and Anderson (2002).

Occupancy Estimation

Model selection results for occupancy estimation are shown in Table 1. The sine and cosine functions for month did not improve model fit of detection probabilities, nor did the percentage of short grass prairie improve estimates of detection probabilities. However the percentage of short grass prairie did provide an important predictor of occupancy (Figure 3) with the logit predictive equation:

$$\text{Occupancy Probability} = \frac{\exp[\hat{\beta}_0 + \hat{\beta}_1(\text{SGP\%})]}{1 + \exp[\hat{\beta}_0 + \hat{\beta}_1(\text{SGP\%})]} \; ,$$

where $\hat{\beta}_0$ = -0.287 (SE = 0.624, 95% CI −1.510 – 0.936) and $\hat{\beta}_1$ = 2.775 (SE = 1.299, 95% CI 0.229 – 5.322).

The estimated occupancy rate using the average amount of short grass prairie found on the 51 grids samples was $\hat{\psi}$ = 0.777 (SE = 0.0786, 95% CI 0.589 – 0.894). When the estimated occupancy was summed across the 51 grids using the observed amount of short grass prairie on each grid, $\hat{\psi}$ = 0.742 (SE = 0.0869, 95% CI 0.572 – 0.912). Finally, the entire population of grids from which the 51 sampled grids were drawn was used to compute the proportion of eastern Colorado occupied by swift foxes: $\hat{\psi}$ = 0.711. The amount of short grass prairie for each of the grids in the population was estimated based on a GIS layer.

Population Estimation

Model selection results for population estimation (Table 2) suggest a behavioral effect in response to initial capture, with capture probabilities a function of month and SGP. Initial capture probabilities (Figure 4) and recapture probabilities (Figure 5) from the minimum AICc model are a function of month through a sin transformation, and SGP.

The mean number of animals estimated per grid for all 51 grids was 4.83 (SE = 1.990, 95% CI 0.933 – 8.735), ranging from 0 to 26.

DISCUSSION

Simulations reported in Finley et al. (2005) reported expected power to detect declines given various combinations of numbers of trapping occasions and numbers of grids trapped. For 50 grids trapped with 3 occasions, their simulation results suggested a SE of about 0.070 for ψ = 0.8. The estimated SEs from this study are slightly greater then this value, likely because of the variation in SGP over the range of the sample. However, the values are close enough to make the simulation results reported in Finley et al. (2005) useful if taken a bit conservatively.

The results from this study concerning the importance of SGP in predicting swift fox occupancy compared favorably with the results obtained by Finley et al. (2005) (Figure 6). Basically, the same relationship of SGP to occupancy was found. However, the minimum AICc model for occupancy in this study was much simpler than that of Finley et al. (2005), mainly because grids were trapped only during the period late August through March when the highest detection probabilities were expected based on Finley et al. (2005) work.

When Finley et al. (2005) used the percentage of short grass prairie for each of their sampled grids to estimate a grid-specific ψ value, the sum of $\hat{\psi}$ values was 56.9 (SE = 4.13), or 56.9 of the 72 grids actually contained foxes ($\hat{\psi}$ = 0.790, SE = 0.0574). Alternatively, they estimated ψ of 0.821 (SE = 0.0659) using the mean (66.9%) of the short-grass prairie habitat for the 72 grids. In either case, their estimates are slightly greater than the values of ψ estimated in this study with the same approaches, but negligibly so when the uncertainty of the estimates is taken into account.

As cautioned in Finley et al. (2005), the mean number of animals estimated per grid cannot be extrapolated to a population estimate for eastern Colorado because the grids attract foxes from some unknown distance outside the trapping grid.

SUMMARY

Comparison of the estimates of the percent of 12-mi^2 grids occupied by swift foxes in eastern Colorado does not appear to have changed since a comparable sample was taken of 72 grids in March, 1995 – January, 1997 (Finley et al. 2005). Using the average percentage of the grids in short grass prairie with the minimum AICc model, the earlier estimate was $\hat{\psi}$ = 0.821 (SE 0.0659), compared to the current estimate of $\hat{\psi}$ = 0.777 (SE = 0.0786). The estimated change is −0.044 (SE = 0.103, 95% CI −0.245 – 0.157). Summing the predicted occupancy values across the sampled grids for the respective studies provides a similar conclusion: Finley et al. (2005) found $\hat{\psi}$ = 0.790 (SE = 0.0574), whereas this study found $\hat{\psi}$ = 0.742 (SE = 0.0869), providing an estimate of the change of −0.048 (SE = 0.104, 95% CI −0.252 – 0.156). These differences are well within the sampling variation of the estimates, and do not suggest a decline in swift fox populations in eastern Colorado.

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______________. 1991. Some practical aspects of a conditional likelihood approach to capture experiments. Biometrics 47: 725–732.

MACKENZIE, D. I., J. D. NICHOLS, G. B. LACHMAN, S. DROEGE, J. A. ROYLE, AND C. A. LANGTIMM. 2002. Estimating site occupancy when detection probabilities are less than one. Ecology 83: 2248–2255.

WHITE, G. C., AND K. P. BURNHAM. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46 Supplement: 120–138.

Prepared by: ________________________

Dr. Gary C. White, Department Fishery & Wildlife Conservation Biology
Colorado State University

Table 1. Occupancy model selection results for 51 swift fox grids trapped in eastern Colorado, August 2004 to February, 2005.

Model	AICc	ΔAICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{p(.) ψ(SGP)}	196.785	0	0.39689	1	3	190.274
{p(sinMonth) ψ(SGP)}	198.882	2.0969	0.1391	0.3505	4	190.012
{p(SGP) ψ(SGP)}	198.891	2.1065	0.13843	0.3488	4	190.022
{p(cosMonth) ψ(SGP)}	199.133	2.3486	0.12265	0.309	4	190.264
{p(.) ψ(.)}	200.412	3.6277	0.0647	0.163	2	196.162
{p(sinMonth+cosMonth) ψ(SGP)}	201.176	4.3916	0.04416	0.1113	5	189.843
{p(T) ψ(.)}	201.242	4.4573	0.04273	0.1077	3	194.731
{p(cosMonth+cosMonth^2) ψ(SGP)}	201.522	4.7372	0.03715	0.0936	5	190.189
{p(t) ψ(.)}	203.449	6.6642	0.01418	0.0357	4	194.579

Table 2. Closed population estimator model selection results for 51 swift fox grids trapped in eastern Colorado, August 2004 to February, 2005.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	No. Par	Deviance
{p(SGP+sinMonth)= c(SGP+sinMonth)+additive effect}	331.785	0	0.4213	1	4	323.666
{p(SGP+sinMonth+ cosMonth)=c(SGP+ sinMonth+cosMonth)+additiv e effect}	333.739	1.9538	0.15861	0.3765	5	323.56
{p(SGP)=c(SGP)+ additive effect}	334.006	2.2207	0.13879	0.3294	3	327.935
{p(sinMonth)= c(sinMonth)+ additive effect}	334.421	2.636	0.11277	0.2677	3	328.35
{p(cosMonth)= c(cosMonth)+ additive effect}	336.195	4.4094	0.04646	0.1103	3	330.124
{p(cosMonth+ sinMonth)= c(cosMonth+ sinMonth)+additive effect}	336.322	4.5372	0.04359	0.1035	4	328.204
{p(.) c(.)}	337.474	5.6892	0.0245	0.0582	2	333.439
{p(T)=c(T)}	337.658	5.8723	0.02236	0.0531	2	333.622
{p(.)=c(.)}	338.619	6.8334	0.01383	0.0328	1	336.607
{p(T)=c(T)+ additive effect}	339.211	7.4255	0.01028	0.0244	3	333.14
{p(t)=c(t)+ additive effect}	339.84	8.0543	0.00751	0.0178	4	331.721
{p(g*t)=c(g*t)}	537.81	206.024	0	0	108	220.762

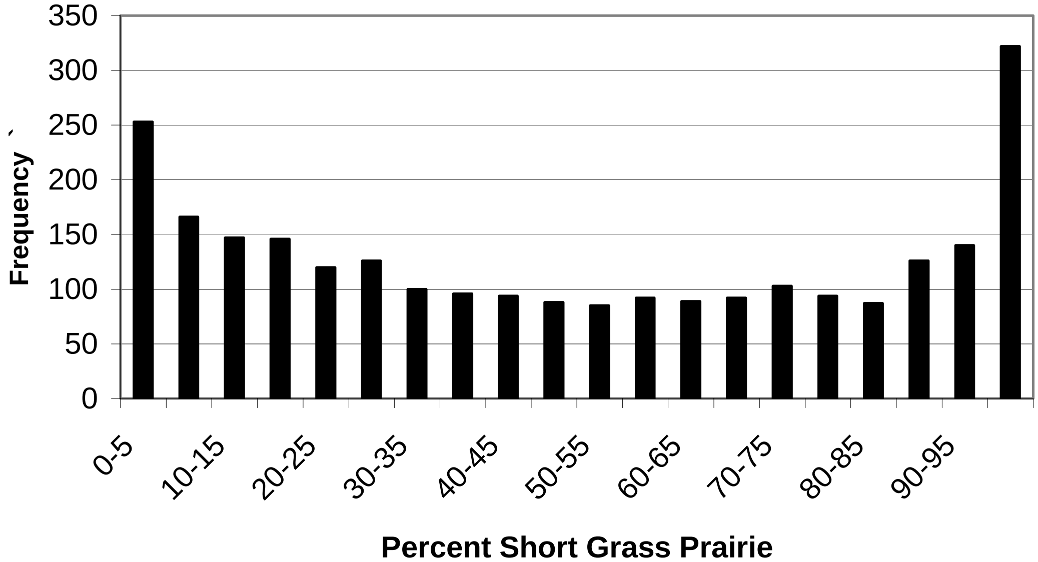

Figure 1. Histogram of percentage of short grass prairie on 12-mi^2 trapping grids comprising the sampling frame for this study.

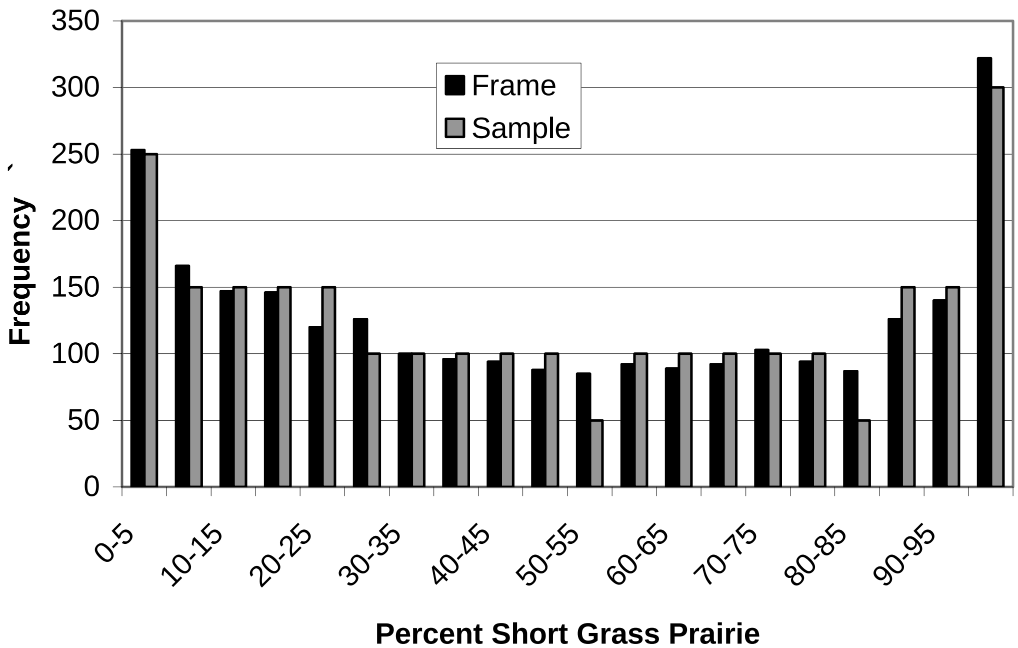

Figure 2. Histogram showing the close relationship between the grids included in the sample compared to the sampling frame. A representative sample relative to the availability of the SGP variable was selected.

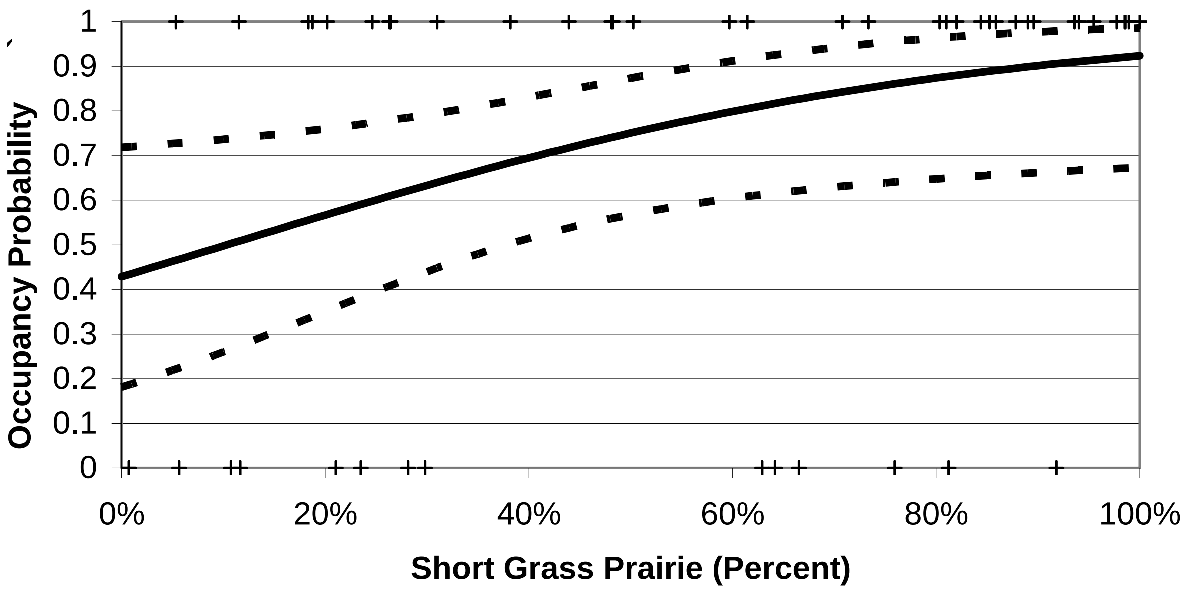

Figure 3. Prediction of the probability of occupancy with 95% confidence intervals as a function of the percentage of short grass prairie on the 12-mi^2 trapping grid. Ticks on the 0 and 1 lines indicate the status of the 51 trapping grids, with 36 of the grids recording foxes captured.

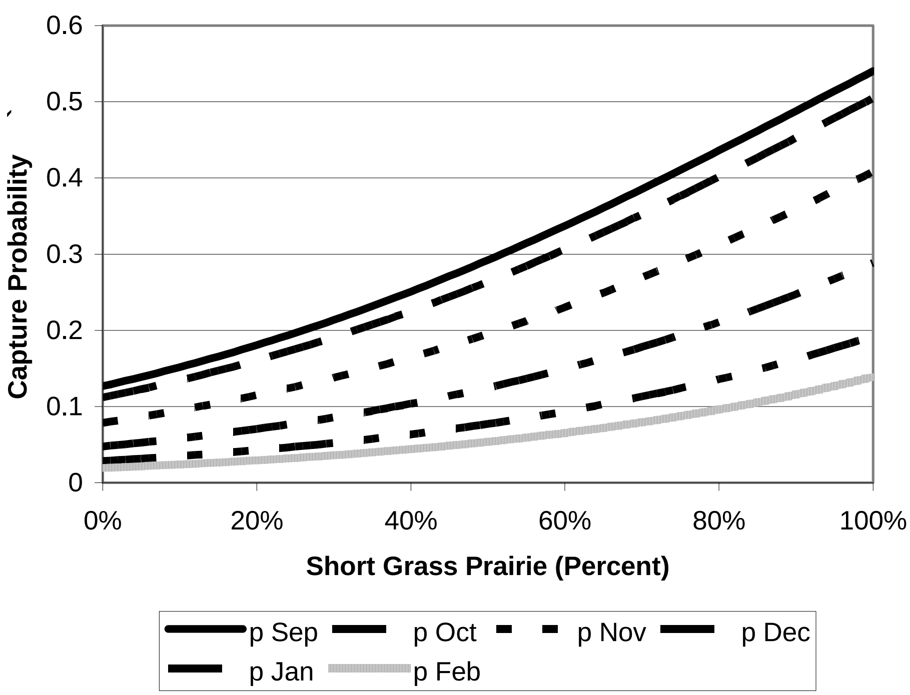

Figure 4. Changes in initial capture probability for swift fox trapped in eastern Colorado on 12-mi^2 grids, August 2004 – February, 2005.

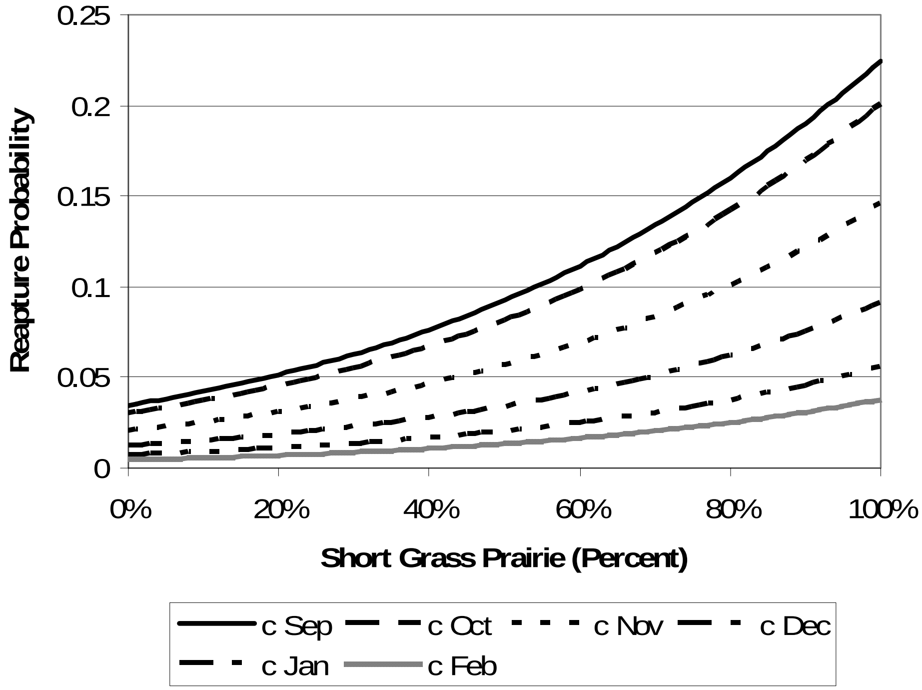

Figure 5. Changes in recapture probability for swift fox trapped in eastern Colorado on 12-mi^2 grids, August 2004 – February, 2005.

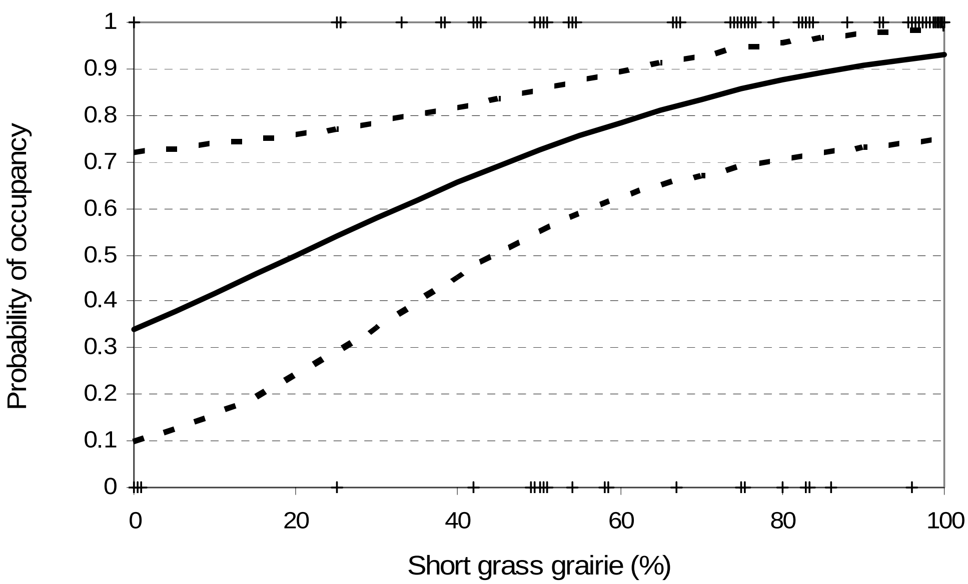

Figure 6. Effect of the percentage of the 12–mi^2 grid consisting of short-grass prairie habitat on the probability of occupancy by swift foxes trapped on 72 grids in eastern Colorado, March, 1995 – January, 1997, for the top-ranked AICc model $\{p(T + \cos(\text{Month}) + \cos2(\text{Month}))\ \psi\ (\text{SGP Proportion})\}$ from Finley et al. (2005). The dashed lines are 95% confidence intervals for the estimated probability of occupancy. Ticks across the 0 and 1 occupancy lines are the observed occupancy values plotted against the percentage of short grass prairie for the 72 grids, with short grass prairie values dithered so that grids would not plot on top of each other.

Colorado Division of Wildlife
July 2004 – June 2005

WILDLIFE RESEARCH REPORT

State of Colorado	: Division of Wildlife
Cost Center 3430	: Mammals Research
Work Package 3002	: Elk Conservation
Task No. 2	: Evaluation of GnRH Vaccine as a Long-term Contraceptive Agent in Female Elk: Effects on Reproduction and Behavior
Federal Aid Project: N/A	:

Period Covered: July 1, 2004 – June 30, 2005

Author: D. L. Baker and J. G. Powers

Personnel: J. Powers, M. Wild (National Park Service), L. Miller, J. Rhyan (National Wildlife Research Center), M. Conner (Utah State University), T. Nett (Colorado State University).

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ABSTRACT

We conducted a pilot experiment to evaluate the potential of GnRH vaccine as a long-term contraceptive agent in female elk. The objectives of this preliminary investigation were to characterize the antibody response of captive female elk to GnRH vaccine, evaluate the effectiveness of dart delivery of the agent, and document the presence and severity of systemic reactions (if any) to the treatment. Intramuscular injection of GnRH vaccine was accomplished in 4 female elk. Serum antibody responses were collected each month beginning in February, 2005 and submitted for analysis. Ultrasound imaging of the injection site was conducted in conjunction with monthly blood collections. Analysis of antibody levels have not been completed, however initial results from ultrasound imaging of vaccine injection sites reveal changes in muscle fiber and muscle tissue echogenicity compared to pre-treatment conditions. All animals show some level of disruption of normal muscle fiber patterns and changes in the quality of muscle tissue. These changes began to appear approximately 2 weeks post-treatment, peaked in severity in April then diminished during July, 2005. Based on results of this trial and similar ongoing investigations with captive white-tailed deer (*Odocoileus virginianus),* we prepared a detailed study plan describing research to evaluate GnRH vaccine as a long-term contraceptive agent in female elk (Appendix I). The objectives of this experiment are to evaluate the effects of this fertility control agent on pregnancy rates, reproductive behavior, and neonatal health and survival. We performed a power analysis to determine the sample sizes needed to detect treatment differences for pregnancy rates and reproductive behavior for captive female elk maintained at the Colorado's Foothills Wildlife Research Facility in Fort Collins, Colorado. Based on this analysis, a sample size of 18-26 elk (equally divided between control and treatment groups) should provide adequate statistical power to detect treatment differences in pregnancy rates and reproductive behavior. A detailed description of hypotheses, rationale, methods, and statistical analyses are provided in this report. The status of publications in process is also provided (Appendix II).

WILDLIFE RESEARCH REPORT

EVALUATION OF GnRH VACCINE AS A LONG-TERM CONTRACEPTIVE AGENT IN FEMALE ELK: EFFECTS ON REPRODUCTION AND BEHAVIOR

DAN L. BAKER

P. N. OBJECTIVE

Evaluate the effects of GnRH vaccine on pregnancy rates, fetal and neonatal growth and development, and reproductive behaviors in captive female elk.

SEGMENT OBJECTIVES

1. Conduct a pilot experiment to evaluate individual animal variation in antibody response to GnRH vaccine and assess any side-effects of treatment.
2. Using results from the pilot experiment prepare a study plan program narrative and submit for internal peer review and extramural funding.
3. Summarize and analyze data from previous fertility control experiments and submit manuscripts to appropriate scientific journals.

INTRODUCTION

Hunting and culling have traditionally been used to regulate ungulate numbers but there are a growing number of situations where these methods are not feasible. Such places include urban and suburban areas where lethal removal is often opposed because of safety concerns or on ethical grounds (Decker and Connelly 1989, McAninch 1993, Wright 1993, McCullough et al. 1997). In addition, there are many conservation areas, and state and national parks where hunting may be inconsistent with other goals of resource management or where it is proscribed by law and policy (Leopold et al.1963, Frost et al.1997, Porter and Underwood 1999). In these situations, fertility control offers a potential alternative for limiting the growth of ungulate populations (Kirkpatrick and Turner 1985, Bomford 1990, Garrott et al. 1993). Additionally, development of fertility control technology may provide resource managers benefits beyond its value as a tool for balancing ungulates and their forage resources. Fertility control may reduce the rate of disease transmission in ungulates by regulating local host densities and pathogen shedding (Rhyan and Drew 2002, Miller et al. 2004). Simulation modeling suggests that, in some situations, fertility control can be as effective as culling in reducing endemic disease or the density of susceptible hosts (Hone 1992, Barlow 1996).

Extensive research has been devoted to developing anti-fertility agents that are safe, effective, reversible and economical (Fagerstone et al. 2002) and models have been developed to represent effects of fertility control on population dynamics of wild ungulates (Garrott and Siniff 1992, Seagle and Close 1996, Hobbs et al. 2000). To date, however, only modest successes have been achieved and a practical and acceptable method of controlling reproduction in free-ranging wildlife populations has not yet been attained.

In previous research, we administered gonadotropin-releasing hormone (GnRH) agonist (leuprolide acetate) in a biodegradable implant to captive and free-ranging female elk and achieved 100% contraception for one breeding season, without significant behavioral or physiological side-effects (Baker et al. 2002,2004). However, despite the demonstrated efficacy and safety of this approach over existing technology, practical application is compromised by the need for annual treatments in fall, prior to the breeding season, a time when capture efficiency is low compared to winter and early spring.

GnRH Vaccine

An alternative approach involves immunization against GnRH. GnRH is a small, 10 amino acid, neuropeptide with an obligatory role in reproduction. It is naturally secreted in a pulsatile pattern from neurons in the hypothalamus and specifically directs gonadotropes in the anterior pituitary gland to synthesize and release luteinizing hormone (LH) and follicle stimulating hormone (FSH). These latter two hormones, in turn, control proper functioning of ovaries in females and testes in males (Hazum and Conn 1998).

To successfully immunize an animal against GnRH, it is necessary to make this endogenous protein appear foreign to the host. Therefore, many copies of the peptide are coupled to the highly immunogenic carrier molecule keyhole limpet hemocyanin (KLH). When combined with a potent adjuvant the GnRH-KLH conjugate stimulates the host's immune system to produce antibodies against GnRH as well as KLH. Anti-GnRH antibodies bind to GnRH in the hypothalamic -pituitary portal vessels and prevent the hormone from attaching to receptors on the gonadotropes. This suppresses secretion of LH and FSH, halting the hormonal cascade that is ultimately responsible for folliculogenesis and ovulation. This condition persists as long as there are sufficient antibodies to bind to all circulating GnRH.

The use of GnRH vaccine as a fertility control agent is not new. It has been administered to a variety of domestic ungulates including horses (Rabb et al. 1990), cattle (Adams and Adams 1986), swine (Meloen et al. 1994), and sheep (Brown et al. 1994). It's use as a contraceptive agent in wild ungulates has been limited, however by the need for multiple initial treatments, annual boosters, and the use of the controversial FCA and FIA to enhance the immune response of the vaccine (Miller et al. 2000*b,* Curtis et al. 2002).

Recently, the impracticality of this approach for wildlife applications has been largely overcome by the development of a new adjuvant by scientists at the National Wildlife Research Center (NWRC) in Fort Collins, Colorado, USA. The alternative adjuvant is thought to be safer and, equally as effective in eliciting an antibody response, as FCA or FIA. The new adjuvant (AdjuVac^{TM}) is derived from a USDA-approved Johne's disease vaccine (Mycopar^{TM}) which has previously been approved for use in food animals by USDA/APHIS(http://www.aphis.usda.gov/ws/nwrc/research/gnrh.html) . A single application of GnRH-KLH and AdjuVac^{TM}) may prove to be a safe, practical, and effective multi-year immunocontraceptive for wild ungulates. This approach has several potential advantages over other methods of contraception. These include:

1) a single treatment may provide long-term (2 + years) of infertility when administered to pregnant animals during winter
2) effectiveness of treatment may be > 90% during the first breeding season following immunization
3) infertility should be reversible
4) the agent should not cause significant behavioral or physiological side-effects
5) the agent should be safe for pregnant animals and the developing fetus
6) the proteinaceous nature of the GnRH-KLH immunogen should eliminate the possibility of passage through the food chain
7) the small volume required for effective contraception should facilitate administration by syringe dart
8) the agent is currently being evaluated for FDA approval as a New Animal Drug and therefore may be available for commercial use in the near future.

Preliminary investigations evaluating GnRH-KLH vaccine in captive wild horses (Killian et al.

2005, in preparation), bison (Miller et al. 2004) and white-tailed deer (Miller et al. 2004, unpublished data) are promising and USDA/APHIS is seeking FDA registration of the new vaccine and adjuvant (GonaCon/AdjuVac^{TM}). However, many unanswered questions must be addressed before this potential contraceptive can be considered an effective and acceptable method of population control in free-ranging elk. Research is needed to evaluate the effectiveness and duration of this approach in elk, the effects on elk reproductive physiology, the effect on elk social structure of removing individuals from the breeding population, and the practicality/feasibility of application in wild populations.

Captive Elk Experiments

Rationale: The efficacy of GnRH-KLH vaccine depends on sufficient stimulation of the immune system and subsequent production of antibodies against this reproductive hormone. Thus, an initial step in assessing the potential of a single application of GnRH-KLH vaccine as a contraceptive agent in elk is to evaluate antibody response to treatment. Such studies have been conducted in wild horses (Killian et al. unpublished data), bison (Miller et al. 2004, in press), and white-tailed deer (Miller et al. unpublished data) but not in elk. Results of these studies indicate that the immunological response to GnRH-KLH vaccine is not uniform across species and highly variable within species. As a consequence, a species specific experiment is required to measure peak antibody response in female elk, time to peak response, and duration of response. Although such titers may not provide a quantitative measure of infertility, their characterization is of interest because sustained elevation of anti-GnRH antibody titers has been consistently associated with infertility in other species. Thus, the primary purpose here was to provide preliminary information on antibody response in elk, to determine optimum sample sizes for future experiments, to assess gross and clinicopathalogical side-effects of treatment (if any), and to evaluate remote delivery of the vaccine.

Objectives: We conducted a controlled pilot experiment with captive elk to:

1) characterize serum antibody response of captive female elk to GnRH-KLH vaccine.
2) evaluate the effectiveness of dart delivery of GnRH-KLH vaccine.
3) evaluate presence and severity of systemic reactions or abscesses (if any) to the GnRH-KLH/AdjuVac^{TM} vaccine treatment
4) determine if vaccination with GnRH/AdjuVac^{TM} causes seroconversion to Johne's disease mycobacteria.

This experiment was conducted at the Colorado Division of Wildlife's Foothills Wildlife Research Facility (FWRF) in Fort Collins, Colorado, USA with the approval of the Colorado Division of Wildlife Animal Care and Use Committee (# 1-2005) and in compliance with U.S. Federal Animal Welfare Act Regulations).

METHODS

We conducted an experiments with 4, non-pregnant, multiparous adult female elk beginning 7 February 2005. These elk were closely monitored into July 2005 to meet initial objectives of the pilot experiment, but the health of these elk and responses to the vaccine will be monitored until 1 August 2007. The captive elk used in this experiment were permanently maintained at FWRF and were trained to repeated handling, weighing, and blood sampling procedures. On the day before treatment (7 February), elk were moved from holding pastures (5 ha) and placed in individual isolation pens. The next day, each elk received a single injection of 1000µ :g of GnRH-KLH conjugate (0.5 ml aqueous solution) emulsified in 1.0 ml of AdjuVac^{TM}, as a water in oil emulsion. The conjugate was be transferred into single use, 1 ml, 13-mm-diameter, barbless darts equipped with gel-collared 32-mm-long needles (Pneu-dart, Williamsport, Pennsylvania, USA).

Prior to darting, individual elk were placed in a handling chute and lightly sedated with xylazine hydrochloride (15-20 mg/animal, IV). This dose allowed the animal to remain standing in the chute and minimized excitation associated with discharge of the dart gun. We examined of the reproductive tract of each elk using rectal palpation and ultrasonographic techniques, collected blood samples (20-30 ml) and measured body weight (∀ 0.5 kg). Elk were remotely injected in the biceps femoris muscle with a dart fired from a CO_2-powered pistol (DanInject™, Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) from a distance of approximately 3 meters. In order to accurately determine the precise dose of GnRH-KLH delivered to each elk, darts were weighed before and after injection. If a dart failed to discharge or only partially injected the prescribed dose, additional darts were fired until the complete dose was delivered to the animal. Once the vaccine had been administered, sedation was reversed with yohimbine (30 mg, IV) (Antagonil®, Wildlife Laboratories, Fort Collins, Colorado, USA) and elk were returned to holding pastures.

One of the elk (F86) used in this experiment was previously used in a *Brucella abortus* Strain 19 vaccination study (1998). It may still retain antibodies or immune modulation relative to this organism that could influence its immune response to the AdjuVac™ portion of the GnRH vaccine. This elk has not shown any evidence of being affected by Johne's disease (*Mycobacterium avium partuberculosis).* However, the AdjuVac™ adjuvant uses small amounts of a remarkably similar killed bacterium (derived from the Johne's vaccine Mycopar™). This could cause seroconversion indistinguishable from Johne's disease.

Pre-vaccination serum was submitted for a large animal biochemistry profile, Johne's disease ELISA, and Strain 19 brucellosis vaccination serology. Elk were monitored for local injection site reactions (swelling, erythema, drainage) on a daily basis for 1 week, and on a biweekly basis for the following 2 months. A second biochemistry profile was submitted if elk showed symptoms of local or systemic inflammation. Ultrasound examination of the injection site may was used to evaluate abscess and granuloma formation.

Serum anti-GnRH antibody production was monitored on a bimonthly basis until peak anti-body titers were determined, then on a bimonthly basis thereafter until termination of the experiment. Once a measurable ($P < 0.05$) decrease in anti-body levels is observed, the need to continue monitoring anti-GNRH antibodies will be reevaluated. Once peak response in each elk has been achieved, a second reproductive examination will be performed to evaluate any changes in ovarian structures.

Analysis: This was a descriptive experiment and no hypotheses were tested. We used descriptive statistics to examine changes in antibody titers over time.

Schedule:

Date	Activity
12 January 2005	Submit study plan for ACUC approval
7 February 2005	Move experimental elk to individual isolation pens
8 February 2005	Perform pre-treatment exams and administer GnRH-KLH conjugate to elk
8 February to 8 March 2005	Intensive health monitoring of elk
February 2005 to August 2006	Ongoing health and anti-GnRH antibody monitoring, and compile and analyze data pertinent to Expt. 2

RESULTS AND DISCUSSION

Intramuscular injection of GnRH vaccine was accomplished in 4 female elk. Serum antibody responses of experimental elk were collected each month beginning in February, 2005 and submitted for analysis. Initial results from ultrasound imaging of vaccine injection sites reveal changes in muscle fiber and muscle tissue echogenicity compared to pre-treatment conditions. All animals show some level of disruption of normal muscle fiber patterns and changes in the quality of muscle tissue. These changes began to appear approximately 2 weeks post-treatment and have not been resolved to date.

SUMMARY

Results of the pilot experiment are incomplete at this time. Initial results suggest that GnRH vaccine can be delivered via intramuscular dart injection. However, until laboratory results are completed, it is unknown if the antibody response of elk to GnRH vaccine will be sufficiently high to suppress fertility. Regardless, injection site reaction to the vaccine is a concern and warrants further evaluation.

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Dan L. Baker, Wildlife Researcher

APPENDIX I

PROGRAM NARRATIVE STUDY PLAN
FOR MAMMALS RESEARCH
FY 2004 – FY 2007

State of: Colorado	: Division of Wildlife
Cost Center: 3430	: Mammals Research
Work Package: 3002	: Elk Conservation
Task No.: 2	: Evaluation of GnRH Vaccine as a Long-Term Contraceptive Agent in Female Elk: Effects on Reproduction and Behavior
Federal Aid Project No.: N/A	:

EVALUATION OF GnRH VACCINE AS A LONG-TERM CONTRACEPTIVE AGENT IN FEMALE ELK: EFFECTS ON REPRODUCTION AND BEHAVIOR

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STUDY PLAN APPROVAL

Prepared by: ______________________________ Date: ______________________

Submitted by: ______________________________ Date: ______________________

Reviewed by: ______________________________ Date: ______________________

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Reviewed by: ______________________________ Date: ______________________
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PROGRAM NARRATIVE STUDY PLAN
FOR MAMMALS RESEARCH

State of : Colorado	:	Division of Wildlife
Cost Center: 3430	:	Mammals Research Program
Work Package: 3002	:	Elk Conservation
Study No.:	:	Evaluation of GnRH Vaccine as a Long-Term Contraceptive Agent in Female Elk: Effect on Reproduction and Behavior

A. STUDY TITLE:

Evaluation of GnRH Vaccine as a Long-term Contraceptive Agent in Female Elk: Effects on Reproduction and Behavior

B. NEED:

Overabundant wild ungulate populations have become a significant problem for natural resource managers in many areas of North America. Unregulated populations can cause adverse effects that are ecological, economic, or political in scope and resolving these problems often requires managing excessive animal numbers (Jewell and Holt 1981, Garrott et al. 1993).

Hunting and culling have traditionally been used to regulate ungulate numbers but there are a growing number of situations where these methods are not feasible. Such places include urban and suburban areas where lethal removal is often opposed because of safety concerns or on ethical grounds (Decker and Connelly 1989, McAninch 1993, Wright 1993, McCullough et al. 1997). In addition, there are many conservation areas, and state and national parks where hunting may be inconsistent with other goals of resource management or where it is proscribed by law and policy (Leopold et al.1963, Frost et al.1997, Porter and Underwood 1999). In these situations, fertility control offers a potential alternative for limiting the growth of ungulate populations (Kirkpatrick and Turner 1985, Bomford 1990, Garrott et al. 1993). Additionally, development of fertility control technology may provide resource managers benefits beyond its value as a tool for balancing ungulates and their forage resources. Fertility control may reduce the rate of disease transmission in ungulates by regulating local host densities and pathogen shedding (Rhyan and Drew 2002, Miller et al. 2004). Simulation modeling suggests that, in some situations, fertility control can be as effective as culling in reducing endemic disease or the density of susceptible hosts (Hone 1992, Barlow 1996).

Extensive research has been devoted to developing anti-fertility agents that are safe, effective, reversible and economical (Fagerstone et al. 2002) and models have been developed to represent effects of fertility control on population dynamics of wild ungulates (Garrott and Siniff 1992, Seagle and Close 1996, Hobbs et al. 2000). To date, however, only modest successes have been achieved and a practical and acceptable method of controlling reproduction in free-ranging wildlife populations has not yet been attained.

GnRH Agonist

Gonadotropin-releasing hormone (GnRH) is an endogenous neuropeptide that has an obligatory role in reproduction. It is naturally secreted in a pulsatile pattern from neurons in the hypothalamus and specifically directs gonadotropes in the anterior pituitary gland to synthesize and release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These latter two hormones, in turn, control proper functioning of the ovaries in females and testes in males (Hazum and Conn 1988).

The chemical structure of endogenous GnRH has been determined (Matsuo et al. 1971) and alterations in the molecule have led to the synthesis of potent GnRH agonist analogs (Karten and Rivier 1986). Long-term treatment with GnRH agonists has been shown to prevent ovulation by decreasing GnRH receptors on gonadotropes, receptor sensitivity to GnRH (Nett et al. 1981), pituitary LH content (Aspden et al. 1996), and by suppressing pulsatile secretion of LH and FSH (D'Occhio et al. 1996).

Agonists of GnRH have been used in domestic animals as fertility agents for controlling ovarian activity, gonadal steroidogeneis, and reproduction (McNeilly and Fraser 1987, Montovan et al. 1990, D'Occhio et al. 2002). In previous research, the GnRH agonist, leuprolide, was administered to captive female elk (*Cervus elaphus)* and mule deer (*Odocoileus hemionus)* in a controlled release bioimplant and achieved 100% infertility for one breeding season, without significant behavioral or physiological side-effects (Baker et al. 2002, 2003, 2004). However, despite the demonstrated efficacy and safety of this approach over existing technology, practical application is compromised by the need for annual treatments in fall, prior to the breeding season, a time when capture efficiency is low compared to winter and early spring.

Immunocontraception

To date, most wildlife contraceptive efforts have been directed toward development of a safe and effective immunocontraceptive vaccine. The immunocontraceptive target antigen that has received the most research and management attention is porcine zona pellucida (PZP). Porcine zona pellucida has been administered experimentally to more than 70 species of wild mammals (Kirkpatrick et al. 1997). This approach relies on host antibodies formed against PZP to block sperm receptor sites on the ovum, thereby preventing fertilization and pregnancy (Dunbar and Schwoebel 1988). The PZP vaccine has been shown to be 85-90% effective in most ungulates, can be administered by syringe dart, is reversible, does not interfere with ongoing pregnancies, and most importantly, the immunogen is proteinaceous and therefore, is not likely to pose a threat to the environment or to non-target species, including humans (Kirkpatrick et al. 1990, Turner et al. 1992, Miller et al. 2000*a*, Kirkpatrick and Turner 2002, Shideler et al. 2002, Naugle et al. 2002).

However, despite these desirable characteristics treatment inefficiency and undesirable side-effects have limited management application of PZP vaccine (Rudolph et al. 2000, Turner and Kirpatrick 2002, Naugle et al. 2002). Specifically, practical application is compromised by the requirement that the target animal must receive two initial injections within 1-2 months of each other (Walter et al. 2002). Second, with the exception of SpayVac™ which encapsulates PZP within a cholesterol/phospholipid formulation (Fraker et al. 2002), effective duration is typically < 1 year; consequently, annual booster inoculations are required (Kirkpatrick et al. 1996; Turner et al. 1996). Third, while no long-term health effects have been reported for animals treated with PZP (Kirkpatrick et al. 1995, Miller et al. 2000*a,* Turner and Kirkpatrick 2002), extended estrous cycling and associated breeding behavior have been reported for white-tailed deer (Turner et al. 1992, 1996; McShea et al. 1997), horses (Plotka et al. 1989), elk (Heilmann et al. 1997), and fallow deer (Fraker et al. 2002). By prolonging the breeding season in males and females, PZP vaccine treatments could result in late pregnancies, parturition beyond the normal early summer period, and unpredictable and abnormal behavioral consequences. Finally, for an effective immune response, the PZP antigen must be administered with an adjuvant - a substance that enhances the specific immune response to the antigen. At present, the most effective adjuvants used with PZP are Freund's complete (FCA) and Freund's incomplete adjuvant (FIA). In some species, however, this combination has been shown to cause severe systemic reactions, chronic pain, and abscesses at the injection site (Anderson and Alexander 1983, Stills and Bailey 1991, Leenaars et al. 1996) and, as a consequence, it is unlikely that the Food and Drug Administration (FDA) will grant approval for the use of PZP vaccine containing these adjuvants. Thus, in the near future, practical application of immunocontraception for wildlife species will depend on development and use of improved vaccines with different adjuvants.

GnRH Vaccine

An alternative to PZP immunocontraception involves immunization against GnRH. To accomplish this, it's necessary to make this endogenous protein appear foreign to the host. Therefore, many copies of the peptide are typically coupled to a highly immunogenic carrier molecule such as keyhole limpet hemocyanin (KLH) (Levy et al. 2004). When combined with a potent adjuvant, the GnRH-KLH conjugate stimulates the host's immune system to produce antibodies against GnRH as well as KLH. Anti-GnRH antibodies bind to endogenous GnRH in the hypothalamic - pituitary portal vessels and prevent the hormone from attaching to receptors on the gonadotropes. This mechanism suppresses secretion of LH and FSH and interrupts the normal cascade of hormonal events that are ultimately responsible for folliculogenesis and ovulation.

The use of GnRH vaccine as a fertility control agent is not new. It has been administered to a variety of domestic ungulates including horses (Rabb et al. 1990, Turkstra et al. 2005), cattle (Adams and Adams 1990), swine (Meloen et al. 1994), and sheep (Brown et al. 1994). However, its use as a contraceptive agent in wild ungulates has been limited by the need for multiple initial treatments, annual boosters, and the use of the controversial FCA and FIA to enhance the immune response of the vaccine (Miller et al. 2000*b,* Curtis et al. 2002).

Recently, however, the impracticality of this approach for wildlife applications has been largely overcome by the development of a new adjuvant by scientists at the National Wildlife Research Center (NWRC) in Fort Collins, Colorado, USA. This adjuvant is thought to be safer than, and equally as effective, as FCA and FIA in eliciting an antibody response. The new adjuvant (AdjuVac™) is derived from a United States Department of Agriculture (USDA)-approved Johne's disease vaccine (Mycopar™) which has previously been approved for use in food animals by the USDA, Animal and Plant Health Inspection Service (APHIS) (http://www.aphis.usda.gov/ws/nwrc/research/gnrh.html). A single application of GnRH-KLH and AdjuVac™ (GonaCon™) has the potential to be a safe, practical, and effective multi-year immunocontraceptive for wild ungulates. As a contraceptive for wildlife, this agent offers the following desirable characteristics:

1) A single treatment should provide long-term infertility (2 + years) when administered either to non-pregnant females prior to the breeding season or to pregnant females during gestation.
2) Treatment effectiveness should be 85-90% the first breeding season.
3) Infertility should be reversible.
4) The agent should be safe for pregnant animals and the developing fetus
5) The agent should not cause significant behavioral or physiological side-effects.
6) The proteinaceous nature of the GnRH-KLH immunogen should eliminate the possibility of passage through the food chain.
7) The small volume required for effective contraception should facilitate administration by syringe dart.
8) The agent is currently being evaluated for FDA approval as a New Animal Drug and therefore, could be available for commercial use in deer and elk.

Preliminary investigations evaluating a single application of GnRH-KLH vaccine (GonaCon™) in captive female wild horses (*Equus caballus*) (Killian et al. 2004), bison (*Bison bison*) (Miller et al. 2004), white-tailed deer (*Odocoileus virginianis*) (Miller et al. unpublished data), California ground squirrels (*Spermophilus beecheyi*) (Nash et al. 2004), New Zealand white rabbits (*Oryctolagus cunniculi*) (Powers et al. in preparation) and domestic male cats (*Felis catus*) (Levy et al. 2004) are promising. All female bison (n = 5) treated with a single injection containing 1800µg GnRH-KLH and AdjuVac™ have remained infertile for 3 breeding seasons (Miller et al. 2004, Rhyan and Miller unpublished data). Similarly, mares (n = 18) treated with either 1800µg or 2800µg GnRH-KLH vaccine have been shown to be 100% infertile after one breeding season (Killian et al. 2004). While these results are encouraging, additional species specific studies are needed to confirm the safety and effectiveness of this contraceptive approach in wildlife. Our goal in this investigation is to conduct controlled experiments with captive elk to investigate the important attributes of this technology prior to management application in free-ranging wild ungulates.

C. OBJECTIVES

1. To evaluate the effective duration of a single dose application of GnRH-KLH vaccine in preventing subsequent reproduction in pregnant elk.
2. To evaluate the effect of GnRH-KLH immunization on serum concentrations of LH and progesterone, corpus luteum (CL) function and viability, and neonatal health and survival.
3. To evaluate the effect of the GnRH-KLH vaccine on breeding behavior of captive elk following a contraceptive treatment applied during the second trimester of pregnancy.
4. To evaluate the physiological side-effects (if any) of GnRH-KLH vaccination on female elk, the developing fetus, and/or neonate.

D. EXPECTED RESULTS OR BENEFITS

The Colorado Division of Wildlife's Strategic Plan (2002-2007), charges the agency with "finding alternatives for game management when hunting is not a viable option" (H-1.5, p 9). One of the performance measures for accomplishing this objective is to develop alternative methods of population control. Successful development and testing of the fertility control technology described in this proposal has the potential to accomplish this objective and provide resource managers with a non-lethal strategy for controlling the growth of some wild ungulate populations when sport hunting is infeasible.

E. APPROACH

Proposed Research:

Working Hypothesis.: In this investigation, we test the general hypothesis that a single intramuscular application of a novel anti-GnRH vaccine in mid-gestation female elk will prevent pregnancy the following breeding season and may prevent pregnancy for two or more subsequent seasons. The exact duration of infertility is unknown but will be determined in this investigation. However, permanent sterility is not anticipated and we expect treated females to eventually return to normal estrous behavior and fertility as antibody titers decline. Furthermore, we don't expect immunization against GnRH-KLH to cause substantial negative physiological or behavioral effects in peri-parturient females or neonatal calves. However, since GnRH-KLH vaccine is expected to suppress reproductive hormones, we predict diminished breeding behaviors in treated female elk compared to controls.

Design: We will test the effects of GnRH-KLH vaccine treatments on pregnancy rates in elk using a Fisher's exact test and evaluate serology of reproductive hormones, anti-GnRH antibody titers, and breeding behavior using a one-way ANOVA for a completely randomized design with repeated measures structure.

Animals: Approximately, 20 adult female elk (2-12 years of age), 2 mature, and 2 sub-adult male elk will be used in this study. Elk are permanently maintained at the Colorado Division of Wildlife's Foothills Wildlife Research Facility (FWRF) in Fort Collins, Colorado. The female elk used in these experiments have been previously trained to repeated handling, weighing, ultrasound, and blood sampling procedures. When not involved in periodic intensive sampling procedures required by this study, elk are maintained in fenced paddocks (5 ha) containing native vegetation and fed a diet consisting of *ad libitum* quantities of grass-alfalfa hay, grain supplement, trace mineral block, and water.

Experiment 1: Effects of GnRH-KLH vaccine on pregnancy rates (objective 1)

Hypothesis:

One year post-vaccination, female elk vaccinated intramuscularly with 1000µg GnRH-KLH + adjuvant (AdjuVac™) during the second trimester of gestation will have significantly (*P* < 0.05) higher anti-GnRH antibody concentrations and lower pregnancy rates than females treated with adjuvant alone.

Rationale:

Vaccination with GnRH-KLH + AdjuVac™ has successfully stimulated sufficient anti-GnRH antibody production to prevent pregnancy in a wide range of species including many ungulate species (Curtis et al. 2002, Miller et al. 2004, and Killian et al. 2004). Of particular importance to our experiment is the ongoing study with white-tailed deer (Miller, personal communication). Preliminary results suggest that multiple year infertility has been achieved with a single treatment of GnRH-KLH vaccine. Since elk and deer are taxonomically similar and share many common ecological, morphological and physiological traits, we expect to observe a similar contraceptive response for both species.

Methods:

Many of the measurements in this experiment (i.e. conception/parturition dates, pregnancy rates, luteal function, and hormone concentrations) will be facilitated by controlling the breeding period of female elk. To do this, we will attempt to synchronize estrous cycles of female elk by using progesterone secreting controlled internal drug release (CIDR) implants (Fennessy et al. 1990, Asher et al. 1993, Lucy et al. 2001). The CIDR implants will be placed in female elk during the last week of August 2005 (see appendix A for detailed protocol). Following CIDR removal (approximately the first week of September 2005), reproductively sound male elk will be released into the same pasture as females (see appendix B for breeding soundness exam protocol). During January 2006, we will determine pregnancy status of all females. Once pregnancy status is determined, pregnant elk will be blocked according to age and body condition, and randomly assigned to either a control or treatment group. We will determine pregnancy rates of treatment and control elk each year thereafter until differences in treatment effects can no longer be detected (*P* > 0.05).

Treatment and control formulations will be applied in the following the manner. On the day of application (approximately mid-January, 2006), animals will be moved from paddocks, weighed (± 0.5 kg), and lightly sedated with xylazine hydrochloride (Rompun; Bayer AG, Leverkusen, Germany; 45-55 mg/animal, IM). This dose should allow animals to remain standing in the handling chute and minimize any possible stress or pain associated with blood collection, reproductive tract examination, ultrasound imaging of injection site, and dart delivery of treatments. All elk will be remotely injected in the area of the biceps femoris muscle with 1 ml, 13-mm-diameter, barbless darts equipped with gel-collared 32-mm-long needles (Pneu-dart, Williamsport, Pennsylvania, USA) fired from a CO_2 – powered pistol (DanInject™, Fort Collins, Colorado, USA). Darts will be fired from approximately 3 m and will contain either GnRH-KLH vaccine + AdjuVac™) (treatment) or AdjuVac™ alone (control). In order to accurately determine the precise dose delivered to each elk, darts will be weighed (0.001g) before and after injection. Once all elk have been treated, sedation will be reversed with yohimbine (30 mg, IV) (Antagonil®, Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) and animals will be returned to holding pastures.

Antibody titers will be measured immediately prior to treatment application and then on a monthly or bimonthly basis until maximal levels are reached. Following peak response, these measurements will be made on a less frequent basis until just prior to subsequent breeding seasons (September 2006, 2007, 2008). At that time, females will be sampled again. Except for this period, monthly sampling will be terminated following October 2006.

The effective duration of GnRH-KLH vaccine in controlling fertility in elk will be determined by comparing pregnancy rates of treated and control elk during January 2007 and 2008. Once pregnancy rates are determined, pregnant elk will be aborted using a combination of prostaglandin $F_{2\alpha}$ and dexamethasone (Bates et al. 1982) (see appendix C for detailed protocol).

Blood sampling procedures for antibody determination, pregnancy rates, hormone concentrations, and serum chemistry and hematology will follow methods previously described. While elk are sedated, blood samples (20-40 ml) will be collected via jugular venipuncture. Serum will be stored at – 70 °C until analyzed for LH, progesterone, and anti-GnRH antibodies. Following the last blood collection, sedation will be reversed and elk returned to paddocks.

Measurements:

Anti-GnRH antibodies will be measured using an enzyme linked immunosorbent assay (ELISA) developed by scientists at the NWRC (USDA/APHIS) and/or using radioimmunoassay (RIA) techniques at Colorado State University's Animal Reproduction and Biotechnology Laboratory (ARBL). The effect of GnRH-KLH vaccine on reproduction will determined in January 2007 and 2008 by measuring pregnancy rates using the presence or absence of pregnancy specific protein B (PSPB) (Huang et al. 2000), rectal palpation (Greer and Hawkins 1967, Hein et al. 1991) and/or ultrasound (Curran et al. 1986).

Analysis:

To determine the sample sizes needed to detect treatment differences for pregnancy rates, we performed a power-based sample size determination for a one-sided Fisher's exact test using a software program (NCSS Pass 2000) (Kang and Kim 2004, Krishnamoorthy and Thompson 2002). For this analysis, we used the highest reported pregnancy rate (approximately 30%) for GnRH-KLH vaccine treated white-tailed deer, 1 year post-vaccination (Curtis et al. 2002). To represent the best and worst case scenarios for control elk, we calculated the sample size requirements for a 90% and 100% pregnancy rate. Based on this analysis, between 18–26 female elk (equally divided between control and treatment groups) should provide an adequate sample size to detect expected differences in pregnancy rates (Table 1). Because the pregnancy rates of the control and treatment groups are expected to be close to 1.0 and 0, respectively, the normal approximation invoked for testing the difference between 2 proportions is not valid (Brown et al. 2001).

Table 1. Estimated sample sizes required to detect differences in pregnancy rates, in control and treatment groups, based on a Fisher's exact test power analysis (NCSS Pass 2000).

Power	Control Group Pregnancy Rate	Treatment Group Pregnancy Rate	Total Sample Size[a]
0.90	1.0	0.0	8
0.90	1.0	0.1	12
0.90	1.0	0.2	14
0.90	1.0	0.3	18
0.90	0.9	0.0	12
0.90	0.9	0.1	14
0.90	0.9	0.2	20
0.90	0.9	0.3	26
0.80	1.0	0.0	8
0.80	1.0	0.1	10
0.80	1.0	0.2	12
0.80	1.0	0.3	16
0.80	0.9	0.0	10
0.80	0.9	0.1	12
0.80	0.9	0.2	16
0.80	0.9	0.3	22

[a] Total sample size assumes an equal number for each group, e.g. 18 means 9 treatment and 9 control female elk.

Experiment 2: Effects of GnRH-KLH vaccine on luteal function and neonatesurvival (objective 2)

Hypothesis:

We are uncertain of the effects of GnRH-KLH vaccine treatments on LH secretion, luteal viability and fetal/neonatal survival. Little conclusive research has been conducted on these relationships in wild or domestic ungulates. Limited evidence suggests that GnRH-KLH-induced suppression of LH and progesterone levels in late gestation are not low enough to terminate pregnancy or negatively affect fetal/neonatal survival.

Rationale:

Corpora lutea (CL) secrete progesterone and are an essential ovarian structure for maintenance of pregnancy in all mammals (Baird 1992). Progesterone is obligatory for early embryonic development and peaks in the blood of pregnant females at different stages of gestation for different species. While progesterone is always produced by the CL in early pregnancy, its role in maintenance of pregnancy varies among species. In some species (i.e. mare, cow, ewe, and women) the CL is not needed for the entire gestational period because the feto-placental unit begins producing sufficient progestins to maintain pregnancy (Squires 1993, Stevenson 1997, Stellflug et al. 1997) In other species (i.e. sow, rabbit, white-tailed deer), surgical removal of the CL will terminate pregnancy regardless of when it occurs during gestation (Plotka et al. 1982, Tomas 1997, Tast et. al 2000).

It is well-documented that progesterone secretion is regulated by several hormones, including LH, which plays a principal role in CL function during both the estrus cycle and pregnancy (Niswender et al. 1976, 1994, Rahe et al. 1980, Farin et al. 1990, Okuda et al. 1999). In contrast, however, studies in cattle (Peters et al. 1994), pigs (Buhr 1987), dogs (Onclin et al. 2000), and to some extent sheep (McNeilly and Fraser 1987) provide evidence that LH may not be essential for all aspects of luteal function, including pregnancy. For wild ungulates, LH suppression due to high doses of the GnRH agonist, leuprolide, were not sufficiently luteolytic to terminate pregnancy when administered to elk during the first 60 days of gestation (Baker et al. 2001). Likewise, bison vaccinated with GnRH-KLH during the second and third trimesters of pregnancy, maintained a viable fetus throughout gestation and delivered healthy calves at parturition (Miller et al. 2004).

In this experiment, elk will be vaccinated with GnRH-KLH at approximately 120 days gestation and should develop sufficient antibody titers to suppress LH by 180-200 days of gestation (Miller et al. 2000b).Because the average gestation period in elk is 255 days (Haigh and Hudson 1993), the animals in this experiment will be in the third trimester of pregnancy before the CL is significantly affected by lack of LH. If the elk respond similarly to cattle and bison they will likely retain the pregnancy despite expected declines in progesterone. Alternatively, if elk are highly sensitive to small changes in progesterone concentrations they may abort the fetus.

Methods:

One day prior to GnRH-KLH vaccine treatments in January, 2006, we will collect blood for antibody titers, LH and progesterone concentrations (Niswender et al. 1969, Niswender 1973), and perform reproductive examinations on all experimental elk. Beginning approximately 4 weeks post-treatment, and in conjunction with scheduled monthly measurements of antibody titers, we will monitor changes in these parameters until 15 April, 2006. Following parturition (approximately June 1-15), we will monitor neonatal health, survival, and growth to 30 days post-parturition. Weaned calves, not needed as replacement animals in other experiments or at other captive research facilities, will be humanely euthanized. At present, we have received a proposal from scientist at USDA/APHIS National Wildlife Research Center to use surplus elk calves in a terminal experiment to develop and test orally active vaccines for managing infectious diseases such as bovine tuberculosis and brucellosis.

Analysis:

We will use descriptive statistical methods to analyze hormonal data. Hormone concentrations, fetal and CL measurements will be reported as arithmetic means ± $\hat{SE}$. We will estimate the correlation coefficient between antibody titers, hormone concentrations, CL measurements, and test whether these relationships are significantly different from zero (Zar 1984). We will compare the differences in growth rates (g/da) of calves born to treatment and control females from birth to 30 days of age using a two sample t-test.

Experiment 3: Effects of GnRH-KLH vaccine on breeding behavior (objective 3)

Hypothesis:

The effectiveness of GnRH-KLH vaccine as a contraceptive agent is dependent on the suppression of ovulation and steroidogeneis. Because GnRH-KLH vaccine is expected to suppress estradiol and therefore sexual receptivity during estrus, we predict that 1) rates of male precopulatory, female precopulatory, and copulatory behavior will be lower for treated females compared to untreated controls, and 2) that rates of general breeding behavior (i.e. herding, establishing and/or defending a harem) will be similar for both treated and untreated females.

Rationale:

In previous research (Baker et al. 2002), we reported that breeding behavior rates of female elk treated with GnRH agonist were not different from those of untreated elk. We attributed this response to basal estradiol concentrations inducing reproductive receptivity in animals that had been exposed to progesterone earlier in the breeding season or during a “silent estrus” (Harder and Moorhead 1980, Asher 1985). However, in the present experiment, GnRH-KLH vaccine should suppress progesterone secretion, estrogen, folliculogenesis and ovulation well in advance of the onset of the September 2006 breeding season. Therefore, there should be no progesterone "priming" effect and no estrous behavior in treated females. Limited observations of male elk engaged in general breeding behaviors related to establishing and/or defending a harem suggest that they don't discriminate between cycling and non-cycling females (Baker et al. 2002). If true, general breeding behavior rates of females treated with GnRH-KLH vaccine in the present experiment should not be different from untreated females.

Methods:

We will test these hypotheses by examining the effects of GnRH-KLH vaccine on reproductive behaviors of female elk during the breeding season (15 September to 31 October 2006). Our experimental unit for analysis will be individual females within each treatment group. On 15 August 2006, two male elk will be placed with treated and control female elk in the same paddocks. All females will be individually identified with color numeric-coded neck collars. Observers will not know which elk are treatments or controls. Behavior observations will be made from a distance of 50-250 m from an elevated tower using binoculars and spotting scope during day, and a spotlight and night vision scope at night. Selected behaviors (Geist 1982) will be recorded using a lap-top computer with a behavioral software program. All-animal all occurrences sampling procedures will be used to sample reproductive behaviors of all experimental animals over a 24 h period (Leaner 1996). Time-of-day sampling periods will be assigned each week using a randomized block design. Each sampling period will consist of at least 2 h of continuous observations. We will group individual sexual behaviors into four general categories (Table 2).Because behavioral interactions are generally short duration (< 30 sec) relative to the sampling interval, we will record the number of occurrences of each behavior rather than the length of time, and calculate rates of sexual interactions as behaviors per animal per hour, then multiply hourly behavior rates by 24 for a daily rate.

Table 2. Elk reproductive behavior and associated behavior categories (Baker et al. 2002).

Behavior Category	**Reproductive Behavior**
General Breeding	Male directed behavior related to establishing, maintaining and defending a group or harem of female elk (i.e. Herding, guarding, tending)
Male Precopulatory	Male courtship behavior directed toward an individual female to induce or detect estrus or ovulation (i.e. urine testing, flehmen, tongue flick, smell or rub)
Female Precopulatory	Female courtship behavior directed toward dominant male to arouse copulatory behavior (i.e. lick and rub male, mount, lordosis, twitch hocks)
Copulatory	Male behavior directed toward a receptive female in estrus (i.e. intromission)

Analysis:

Based on the sample sizes required to detect differences in pregnancy rates (Table 1), we conducted a simulation to estimate the power to detect differences in behavior rates. To complete this simulation, we bootstrapped data from a previous study which examined the effects of an alternate fertility control agent, leuprolide, on female elk reproductive behavior during the breeding season (Baker et al. 2002). Each sample was run through Proc Mixed (SAS 1996) using repeated measures mixed effects structure. The following parameters were used to estimate power based on total experimental sample sizes of 18, 20, or 26 female elk (Table 3).

1. Male pre-copulatory behavior rate was previously shown to be higher than other reproductive behavior rates (Baker et al. 2002). Consequently, this measurement will likely be the most sensitive to detection of treatment effects. Therefore, we used the previously reported male pre-copulatory behavior rates to estimate power for our simulation.
2. The peak of breeding season is approximately one month in length. Therefore, we estimated power using 60 total observation periods. [4wks x 3 observation periods/da x 5 da/wk = 60 obs. periods]

3. We estimated power for 3 different sample sizes using 60 observation periods and bootstrapping data from the previous leuprolide experiment. Ten control elk were randomly selected (with replacement) from the 5 control female elk of the previous leuprolide experiment (thus some elk were used multiple times in a sample). The behavioral response (male precopulatory behavior rate) for each elk was recorded; thus a complete sample consisted of 10 behavior rates for each of the 60 observation periods. Behavior data from control elk was considered the benchmark for comparison. Hence, to estimate power for treatment elk in the current experiment, we followed the same procedure except that the response was multiplied by the effect size. To represent a 50% decrease in the male precopulatory rate directed toward treatment elk, we multiplied the control response by 0.5.
4. Although our behavioral hypothesis predicts that treated female elk will have reduced reproductive behaviors compared to controls, we also estimated sample size for the possibility of increased behavior rates. Thus, we estimated sample sizes for a 75% and 50% reduction in male precopulatory behavior rates toward treated female elk compared to controls, as well as a 50% increase in male precopulatory behavior rates.
5. Power results are based on the number of times an effect was detected during 100 simulations. Because the variance is larger for higher behavior rates, there is less power to detect a 50% increase compared to a 50% decrease. From this analysis we conclude that a sample size of 20; 10 treatment and 10 control animals, and 60 observation periods would provide adequate power (>90%) to detect most of the differences in reduced reproductive behavior rates as well as reasonable power (>75%) to detect a 50% increase in behavior rates between treated and untreated elk.

Table 3. Power results for detecting differences in male precopulatory behavior rates directed toward treated and untreated female elk based on 100 simulations and 60 observation periods.

Difference Between Treatment and Controls[a]	Total Sample Size[b]	Power α=0.05	Power α=0.10
0.25	18	1.00	1.00
0.25	20	1.00	1.00
0.25	26	1.00	1.00
0.50	18	0.99	0.99
0.50	20	1.00	1.00
0.50	26	1.00	1.00
1.50	18	0.70	0.81
1.50	20	0.76	0.84
1.50	26	0.92	0.95

[a] Effect size
[b] Total sample size assumes an equal number for each group, e.g. 18 means 9 treatment and 9 control female elk.

Experiment 4: Effects of GnRH-KLH vaccine on maternal behavior, neonatal survival and growth, blood chemistry and hematology (objective 4)

Hypothesis:

GnRH-KLH vaccine treatments will not result in significant secondary negative behavioral or physiological side-effects in female elk.

Rationale:

To date, the GnRH-KLH vaccine formulated with AdjuVac™ has produced few reported behavioral or physiological side-effects in any species in which it has been tested (Levy et al. 2004, Miller et al. 2004, Killian et al. 2004). However, it's not clear from these studies how extensively the side-effects of this agent have been evaluated. In this investigation, we will evaluate the effects of GnRH-KLH vaccine on maternal/neonatal behavior, neonatal growth and development, serum biochemistry, and injection site reactions.

Methods:

Injection site reactions. On the day prior to treatment application (early January 2006) and while elk are lightly sedated (see pages 6-7 for details), we will perform ultrasound examination of the area of the expected injection site. After dart delivery of the vaccine, we will grossly monitor the injection site on a daily basis for one month for signs of inflammation or drainage. In addition, we will use ultrasound imaging each month for 6 months in conjunction with scheduled animal handling and blood collections to monitor changes in muscle echogenicity that would indicate sub-clinical abscesses or granuloma formation.

Blood Chemistry and hematology. The physiological side-effects of GnRH-KLH treatment will be assessed by comparing serum chemistry, hematology, and body weight dynamics of treated and control elk. Blood samples will be collected in conjunction with previously described measurements just prior to GnRH-KLH vaccination and one week post-vaccination for evidence of short-term inflammation or infection. At three months post-vaccination, additional blood will be collected and analyzed for biochemistry profiles and evidence of abnormal organ function. These samples will be submitted for analysis to Colorado State University, Veterinary Teaching Hospital, Clinical Pathology Laboratory, Fort Collins, Colorado for analysis.

Maternal Bonding, neonatal survival and growth. We will compare maternal/neonatal bonding and neonatal survival and growth of treated and control female elk for 30 days post-parturition during approximately 1 June to 1 July 2006. Parturition behavior of elk will be monitored daily beginning in late May and early June. We will document evidence of dystocia for each adult female, calf birth weight and health, acceptance or rejection by the dam, and growth to 30 days of age. For the purpose of this experiment, we assume that calf survival after 30 days is no longer a function of GnRH-KLH vaccine treatment and multiple factors other than maternal bonding will influence neonatal survival and body weight dynamics.

Analysis:

Means and standard errors of blood parameters, and neonatal growth rates will be estimated using least–squares ANOVA. Hypothesis tests will be based on type III generalized equations that account for correlations in repeated measures.

Project Schedule:

Date	Activity
1 May – June 2005	Submit study plan for CDOW peer review and ACUC approval
1 Sept 2005	Semen evaluation and CIDR's in all experimental female elk.
7 Sept 2005	Remove CIDR and combine males and females.
1 Jan 2006	Determine pregnancy status of females and assign to experimental groups.
1 Jan 2006	Apply contraceptive and monitor short term health effects.
1 Feb – Sept. 2006	Monitor antibody titers of experimental elk.
1 Feb – June 2006	Monitor hormone levels
1 June – July 2006	Monitor birth rates, calf survival, calf weights and cow/calf behavior.
15 Sept. 2006 – 31 Oct. 2006	Evaluate reproductive behavior
Jan 2007	Evaluate pregnancy rates 1 year post- vaccination.
Jan 2008	If funding is available, evaluate pregnancy rates 2 year post-vaccination
Jan 2009	If funding is available, evaluate pregnancy rates 3 year post-vaccination and/or reversibility of contraceptive treatments.
Mar – July 2009	Analyze data and prepare final report.

Budget: This research proposal has been submitted to the Morris Animal Foundation for possible funding during the period June 2006 to Jan 2008.

Category	2005-'06	2006-'07	Total
Personal Services			
1. Co-Investigator(s)	0	0	0
2. Biometrician	5,000	2,500	7,500
3. Wildlife Technicians (TBA)	6,675	11,175	17,850
Total Salaries and Wages	11,675	13,675	25,350
Operating Supplies & Services			
1.Hormone serology			
LH analysis (240 x $20)	3,600	1,200	4,800
Progesterone (240 x $20)	3,600	1,200	4,800
PSPB (40 x $25)	500	500	1,000
2. GnRH Antibody Assays (360 x $12)	2,160	2,160	4,320
3. Biochemistry profile and CBC's (40 x $40)	1,600	0	1,600
4. Miscellaneous veterinary supplies	1,000	1,000	2,000
Total Supplies & Expenses	12,460	6,060	18,520
Animal Maintenance			
Total Animal Care	5,760	5,760	11,520
Subtotal of All Categories	29,895	25,495	55,390
*Maximum of 8% - Indirect Costs (University Overhead)	2,391	2,039	4,430
Grant Total	**32,286**	**27,534**	**59,820**

F. LOCATION:

This study will be conducted at the Colorado Division of Wildlife's Foothills Wildlife Research Facility in Fort Collins, Colorado, USA.

G. RELATED FEDERAL AID PROJECTS: N/A

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APPENDIX II

BAKER, D. L., M. A. WILD, M. M. CONNER, H. B. RAVIVARAPU, R. L. DUNN, AND T. M. NETT. 2005. Evaluation of a remotely delivered formulation of leuprolide acetate as a contraceptive agent in female elk (*Cervus elaphus nelsoni).* Journal of Wildlife Diseases 41: in press.

Abstract: Practical application of fertility control technology in free-ranging wild ungulates often requires remote delivery of the contraceptive agent. The objective of this investigation was to evaluate the potential of remote delivery of leuprolide acetate for suppressing fertility in female elk (*Cervus elaphus nelsoni)*. Fifteen captive adult female elk were randomly allocated to one of three experimental groups. Six elk were injected intramuscularly with a dart containing leuprolide, and the remaining nine elk received the same formulation without leuprolide. We determined pregnancy rates, suppression of luteinizing hormone (LH) and progesterone concentrations, and reversibility of treatments during 1 August 2002 to 3 September 2003. Leuprolide formulation caused a decrease in concentrations of LH and progesterone, temporary suppression of ovulation and steroidogenesis, and effective contraception (100%) for one breeding season. These results extend the practical application of this contraceptive agent to include dart delivery, where in the absence of such technology, wild elk must first be captured and restrained prior to treatment.

BAKER, D. L., M. A. WILD, M. M. CONNER, M. D. HUSSAIN, R. L. DUNN, AND T. M. NETT. 2006. Evaluation of leuprolide as a contraceptive agent in free-ranging elk in Rocky Mountain National Park, Colorado. Journal of Wildlife Management (in preparation).

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INSLERMAN, R. A., J. E. MILLER, D. L. BAKER, J. E. KENNAMER, R. CUMBERLAND, B. STINSON, P. DOERR, AND S. J. WILLIAMSON. 2005. The Wildlife Society Technical Review Committee on Baiting and Artificial Feeding of Game Wildlife Species (in preparation).

Colorado Division of Wildlife
June 2004 – July 2005

WILDLIFE RESEARCH REPORT

State of	Colorado	:	Division of Wildlife
Cost Center	3430	:	Mammals Research
Work Package	3003	:	Predatory Mammals Conservation
Task No.	1	:	Puma Population Structure And Vital Rates On The Uncompahgre Plateau, Colorado
Federal Aid Project:	N/A	:	

Period covered: July 1, 2004―June 30, 2005

Author: K. A. Logan.

Personnel: S. Waters, T. Murphy, K. Crane, T. Mathieson, M. Caddy, and T. Smith of CDOW, J. Bauer of Colorado Cooperative Fishery and Wildlife Research Unit, J. Kane of U.S.D.A. Wildlife Services, volunteers, cooperators including: private landowners, U.S. Forest Service, Bureau of Land Management, and Colorado State Parks, with project support received from The Howard G. Buffett Foundation and Safari Club International Foundation.

ABSTRACT

To begin conducting research on puma population characteristics and dynamics on the Uncompahgre Plateau, public meetings were held, puma hunting regulations were altered for an experimental design, and a study plan was developed and approved along with animal care and handling procedures. Field research began on December 2, 2004. From December 2, 2004 to July 22, 2005 fifteen puma were captured, sampled, tagged and released, including 7 adult pumas (3 males, 4 females) and 8 cubs (3 males, 5 females). Three other pumas were captured with the aid of dogs, but were released without sampling or tagging for safety reasons. One adult female puma was hit and killed by a car on highway 62 at the southern boundary of the study area. The 7 adult pumas wore GPS collars that yielded 355 to 779 locations per puma. GPS locations indicated 139 clusters that were investigated. Prey use was found at 112 clusters, with mule deer (n = 61) and elk (n = 48) comprising the most important items. Tissue samples collected from all puma handled will be used for proposed research on laboratory and field methods to estimate puma numbers using DNA mark-recapture methods. Puma GPS locations will also be used in proposed efforts to develop and test puma habitat suitability models and maps. Information on evaluations of the GPS collar technology and findings at GPS clusters will be used to develop proposed research on puma-prey relationships on the Uncompahgre Plateau.

WILDLIFE RESEARCH REPORT

PUMA POPULATION STRUCTURE AND VITAL RATES ON THE UNCOMPAHGRE PLATEAU, COLORADO

KENNETH A LOGAN

P. N. OBJECTIVE

Quantify puma population sex and age structure; estimate puma population vital rates, including: reproduction rates of females, age-stage survival rates, and immigration and emigration rates; quantify agent-specific mortality rates— all to improve the Colorado Division of Wildlife's (CDOW) model-based approach to managing puma in Colorado.

SEGMENT OBJECTIVES

1. Hold public meetings and contact individuals to inform citizens in the area of Uncompahgre Plateau about the CDOW desires to conduct the puma research.
2. Obtain needed regulations from the Wildlife Commission for experimental research on the study area.
3. Develop a peer-reviewed study plan that is authorized by the Leader of Mammals Research in the CDOW. Develop proper procedures for the capture, restraint, handling, and sampling of puma for research which are authorized by the CDOW Animal Care and Use Committee.
4. Begin quantifying puma population sex and age structure.
5. Begin process of estimating female puma reproduction rates.
6. Begin process of estimating puma sex and age-stage survival rates.
7. Begin process of estimating agent-specific mortality rates.
8. Begin gathering quantitative data on puma movements for the development of sampling methods for direct and DNA-genotype mark-recapture population estimates. Begin gathering puma tissue samples for individual puma genotyping procedures.
9. Evaluate other data sources that could come from this research that might be developed into other puma research relevant to CDOW biologists and managers.

INTRODUCTION

Colorado Division of Wildlife managers need reliable information on puma biology and ecology in Colorado to develop sound management strategies that address diverse public values and the CDOW objective of actively managing puma while "achieving healthy, self-sustaining populations"(CDOW 2002-2007 Strategic Plan:9). Although 4 puma research efforts have been made in Colorado since the early 1970s and puma harvest data is compiled annually, reliable information on certain aspects of puma biology and ecology, and management tools that may guide managers toward effective puma management is lacking.

Mammals Research staff held scoping sessions with a number of the CDOW's wildlife managers and biologists. In addition, we consulted with other agencies, organizations, and interested publics either directly or through other CDOW employees. In general, CDOW staff in western Colorado highlighted concern about puma population dynamics, especially as they relate to their abilities to manage puma populations through regulated sport-hunting. Secondarily, they expressed interest in puma-prey interactions. Staff on the Front Range placed greater emphasis on puma-human interactions. Staff in both eastern and western Colorado cited information needs regarding effects of puma harvest, puma population monitoring methods, and identifying puma habitat and landscape linkages. Management needs identified by CDOW staff and public stakeholders form the basis of Colorado's puma research program, with multiple lines of inquiry (i.e., projects):

Improve our ability to manage puma hunting with enhanced scientific bases, strategies, and tools

- Puma population characteristics (i.e., density, sex and age structure).
- Puma population dynamics and vital rates (i.e., birth rates, survival rates, emigration rates, immigration rates, population growth rates).
- Field methods and models for assessing and tracking changes in puma populations.
- Relative vulnerability of puma sex and age classes to hunter harvest.

Improve our understanding of puma habitat needs and interrelationships of puma management units

- Puma habitat use, movements, and use of landscape linkages.
- Puma recruitment patterns (i.e., progeny, immigration, emigration).
- Models for identifying puma habitat and landscape linkages.

Improve our understanding of the puma's role in the ecology of other species

- Relationships of puma to mule deer, elk, and other natural prey.
- Relationships of puma to species of special concern, e.g., desert bighorn sheep.

Improve our understanding of puma-human interactions and abilities to manage them

- Behavior of puma in relation to people and human facilities.
- Puma predation on domestic animals.
- Effects of translocating nuisance pumas.
- Effects of aversive conditioning on pumas.

While all projects cannot be addressed concurrently, understanding their relationships to one another is expected to help individual projects maximize their benefits to other projects that will assist the CDOW to achieve its strategic goal in puma management (Fig.1).

Management issues identified by managers translate into researchable objectives, requiring descriptive studies and field experiments. Our goal is to provide managers with reliable information on puma population biology and to develop useful tools for their efforts to *adaptively manage* puma in Colorado to maintain healthy, self-sustaining populations.

The highest-priority management needs are being addressed with this intensive population study that focuses on puma population dynamics using sampled, tagged, and GPS/radio-collared puma. Those objectives include:

1. Describe and quantify puma population sex and age structure.
2. Estimate puma population vital rates, including: birth rates, age-stage-specific survival rates, emigration rates, immigration rates.
3. Estimate agent-specific mortality rates.
4. Improve the CDOW's model-based management approaches with Colorado-specific data from objectives 1—3. Consider other useful models.

Concurrently with the tasks associated with the objectives above, significant progress will be made toward a 5th objective, which will initially be subject to *pilot study*— develop methods that yield reliable estimates of population abundance (i.e., numbers and density) and attendant annual population growth rates, such as, direct capture-resight, and DNA genotype capture-recapture.

TESTING ASSUMPTIONS AND HYPOTHESES

Hypotheses associated with main objectives 1—5 of this puma population research are structured to test assumptions guiding puma management in Colorado.

1. Recreational puma hunting management in Colorado Game Management Units (GMUs) is guided by a model to estimate allowable harvest quotas to achieve one of two puma population objectives: 1) maintain puma population stability, or 2) cause puma population decline (CDOW, Draft L-DAU Plans, 2004). Basic model parameters are: puma population density, sex and age structure, and annual population growth rate. Parameter estimates are currently chosen from literature on studies in western states that are deemed to provide reliable information. Background material used in the model assumes a moderate annual rate of growth of 15% (*i.e.,* $\lambda = 1.15$) for the adult and subadult puma population (J. Apker, Carnivore Management Specialist, CDOW, Monte Vista). This assumption is based upon information with variable levels of uncertainty (e.g., small sample sizes, data from habitats dissimilar to Colorado). The key assumption is that the CDOW can manage puma population growth through recreational hunting: for a stable puma population hunting removes the annual increment of population growth (i.e., as estimated from estimates of population density, structure, and λ); for a declining population, hunting removes more than the annual increment of population growth. Parameters influencing λ include population density, sex and age structure, female age-at-first-breeding, age-specific natality, sex- and age-specific survival, immigration and emigration. A descriptive study will ascertain these population parameters in an area that appears typical of puma habitat in western Colorado and will yield defensible population parameters based upon contemporary Colorado data. This study will be conducted in a 5-year *reference period* (i.e., absence of recreational hunting) to allow puma life history traits to interact with the main habitat factors that appear to influence puma population growth (e.g., prey availability and vulnerability, Pierce et al. 2000, Logan and Sweanor 2001). Contingent upon results in the *reference period*, a subsequent 5-year *treatment period* is planned. The *treatment period* will involve the use of controlled recreational hunting to manage the puma population into a decline phase.

H_{1a}: Population parameters measured during a 5-year *reference period* (in absence of recreational puma hunting) in conifer and oak communities with deer, elk and other prey populations typical of those communities in Colorado will yield an estimated annual adult plus subadult population growth rate that will match or exceed $\lambda = 1.15$, which is currently assumed in the CDOW's model-based management.
H_{1aA}: Population parameters measured during a 5-year *reference period* (absence of recreational puma hunting) in conifer and oak communities with deer, elk and other prey populations typical of those communities in Colorado will yield an estimated annual adult plus subadult population growth rate that will be substantially lower (i.e., ≥50% lower, $\lambda \leq 1.075$) than the assumed $\lambda = 1.15$.
H_{1b}: Population parameters during a 5-year *treatment period* (controlled puma hunting) will differ substantially from those measured during the preceding 5-year *reference* period (hunting closure) and will yield an estimated annual adult plus subadult population growth rate that will be approximately $\lambda=0.8$ for at least the first 2 years of the *treatment period*. Hunting-caused mortality will be strongly additive, and will require removal of the annual growth increment (of adults plus subadults) plus 20% (e.g., assume $\lambda = 1.15$, so, $0.15 \times 0.2 + 0.15 = 0.18$; $0.18 \times 100 = 18\%$ annual harvest of adults plus subadults).
H_{1bA}: Population parameters during a 5-year *treatment period* (controlled puma hunting) will not differ substantially from those measured during the preceding 5-year *reference* period (hunting closure), and the adult plus subadult population will not decline on average as a result of hunting mortality. Hunting-caused mortality, reproduction, immigration, and emigration might be compensatory.

2. Considering limitations (i.e., methods, number of years, assumption violations) to the Colorado-specific studies on puma densities cited above (Currier et al. 1977, Anderson et al. 1992, Koloski 2002), managers assume that puma population densities in Colorado are within the range of those quantified in more intensively studied populations in Wyoming (Logan et al. 1986), Idaho (Seidensticker et al. 1973, Alberta (Ross and Jalkotzy 1992, and New Mexico (Logan and Sweanor 2001). The CDOW assumes density ranges of 2.0—4.6 puma/100 km^2 to extrapolate to Data Analysis Units to guide the model-based quota-setting process. Likewise, managers assume that the population sex and age structure is similar to puma populations described in the intensive studies. Using capture, mark, re-capture techniques developed and refined during the study to estimate the puma population, the following will be tested:

H_{2a}: Puma densities during the 5-year *reference period* (absence of recreational puma hunting) in conifer and oak communities with deer, elk and other prey populations typical of those communities in Colorado will vary within the range of 2.0—4.6 puma/100 km^2 and will exhibit a similar sex and age structure to puma populations in Wyoming, Idaho, Alberta, and New Mexico.

3. The increase and decline phases of the puma population make it possible to test hypotheses related to shifts in the age structure of the population which have been linked to harvest intensity in Wyoming and Utah.

H_{2b}: The puma population on the Uncompahgre Plateau study area will exhibit a young age structure after hunting prohibition at the beginning of the *reference period*. During the 5 years of hunting prohibition, greater survival of independent puma will cause an older age structure in harvest-age puma (i.e., adults and subadults) as suggested by the work of Anderson and Lindzey (2005) in Wyoming and Stoner (2004) in Utah.
H_{2c}: As hunting is re-instated in the *treatment period*, the age structure of harvested puma and the harvest-age puma in the population will vary as observed by Anderson and Lindzey (2005) in Wyoming and Stoner (2004) in Utah.

Desired outcomes and management applications of this research include:
1. Quantification of variations in puma population density, sex and age structure, growth rates, vital rates, and an understanding of factors affecting them will aid adaptive puma management by yielding population parameters useful for estimating puma population abundance, evaluation of management alternatives, and effects of management prescriptions.
2. Testing assumptions about puma populations, currently used by CDOW managers, will help those managers to biologically support and adapt puma management based on Colorado-specific estimated puma population characteristics, parameters, and dynamics.
3. Methods for estimating puma abundance (capture-mark-recapture) of known reliability will allow managers to "ground truth" modeled populations and estimate effects of management prescriptions designed to achieve specified puma population objectives in targeted areas of Colorado. Ascertaining puma numbers and densities during the project will require development of reliable monitoring techniques based on capture-mark-recapture methods and models. Potential methods include direct and DNA genotype capture-recapture. Study plans to develop and test feasible field and analytical methods will be developed in the future after we have learned the logistics of performing those methods, after we have preliminary data on puma demographics and movements which will inform suitable sampling designs, and when we have adequate funding.
4. This information will be disseminated to citizen stakeholders interested in pumas in Colorado, and thus contribute to informed public participation in puma management.

STUDY AREA

The study area for the puma population research is on the Uncompahgre Plateau (in Mesa, Montrose, Ouray, and San Miguel Counties, Fig. 2). The study area includes about 2,253 km^2 (870 $mi.^2$) of the southern halves of Game Management Units (GMUs) 61 and 62, and about 155 km^2 (60 $mi.^2$) of the northern edge of GMU 70 (between state highway 145 and San Miguel River). The area is bounded by state highway 348 at Delta, 25 Mesa road and Forest Service road FS503 to Nucla, state highway 97 to state highway 141 to state highway 145 to Placerville, state highway 62 to Ridgeway, U.S. highway 550 to Montrose, and U.S. highway 50 to Delta.

The study area seems typical of puma habitat in Colorado that has vegetation cover that varies from the pinon-juniper covered foothills starting from about 1,700 m elevation to the spruce-fir and aspen forests growing to the highest elevations of about 3,000 m. Mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*) are the most abundant wild ungulates available for puma prey. There are cattle and domestic sheep raised on summer ranges on the study area. Year-round human residents live along the eastern and western fringe of the area, and there is a growing summer residential presence especially on the southern end of the plateau. A highly developed road system makes the study area well accessible for puma research efforts. A detailed description of the Uncompahgre Plateau is in Pojar and Bowden (2004).

METHODS

Reference and Experimental Treatment Periods

This research is structured in two 5-year periods: a *reference period* (years 1—5) and a *treatment period* (years 6—10). The *reference period* is expected to cause a population increase phase. The *treatment period* will be managed to cause a population decline phase. In both phases, puma population structure, and vital rates will be quantified, and some management assumptions and hypotheses regarding population dynamics will be tested. Contingent upon results of pilot studies, we will also estimate puma numbers, population growth rates, evaluate enumeration methods, and test other hypotheses (Logan 2004).

The *reference period*, without recreational puma hunting as a major limiting factor, is consistent with the natural history of the current puma species in North America which evolved life history traits during the past 10,000—12,000 years (Culver et al. 2000) that enable puma to survive and reproduce (Logan and Sweanor 2001). In contrast, puma hunting, with its modern intensity and ingenuity, might have influenced puma evolution in western North America for the past 100 years. Hence, the *reference period*, years 1—5, will provide conditions where individual puma in this population (of estimated sex and age structure) express life history traits interacting with the environment without recreational hunting as a limiting factor. Theoretically, the main limiting factors will be catchable prey abundance (Pierce et al. 2000, Logan and Sweanor 2001). This should allow researchers to understand basic system dynamics *before* the treatment (i.e., controlled recreational hunting). In the *reference period*, all puma in the study area will be protected, except for individual puma that might be involved in depredation on livestock or human safety incidents. In addition, all radio-collared and ear-tagged puma that range in a buffer zone, that includes the northern halves of GMUs 61 and 62, will be protected from recreational hunting.

The *reference period* will allow researchers to quantify baseline demographic data on the puma population to estimate parameters for the CDOW's model-based approach to puma management. Moreover, it will allow researchers to develop and test puma enumeration methods when population growth is known to be in one direction— increasing. Without the hunting closure, pilot data for enumeration methods could be confounded by not knowing if the population was increasing, declining, or stable. The *reference period* will also facilitate other operational needs (because hunters will not be killing the animals) including the marking of a large proportion of the puma population for capture-mark-recapture estimates, and the gathering of movement data from GPS-collared puma to help formalize exact sampling designs for enumeration methods.

During the *treatment period*, years 6—10, experimentally structured recreational puma hunting will occur on the same study area with the intent of causing a decline phase in the puma population by using management prescriptions structured from information learned during previous years. Using recreational hunting for the treatment is consistent with the CDOW's objectives of manipulating natural tendencies of puma populations, particularly survival, to maintain either population stability or population suppression (CDOW, Draft L-DAU Plans, 2004). Theoretically, puma survival will be influenced mainly by recreational hunting, which will be quantified by agent-specific mortality rates of radio-collared puma. The portion of adults and subadults in the population will be reduced by approximately 20% in year 6 and 20% more in year 7. The 20% change was identified by Division managers that requested enumeration tools that might detect 20% changes in puma populations. For managers, detecting the magnitude of puma population decline phases is probably more important that detecting the magnitude of population increase phases. This will also allow quantification of puma population characteristics and vital rates and initial tests of enumeration methods during a decline phase.

Additional reductions may be made to test enumeration methods and other hypotheses that may be related to effects of hunting (i.e.,: relative vulnerability of puma sex and age classes to hunting, variations in puma population structure due to hunting) and puma-prey interactions (i.e., lines of research identified in the Colorado Research Program, Fig. 1). Those decisions can be made later in project development and as late as years 8—10. The killing of tagged and collared puma during the *treatment period* will not hamper operational needs (as it would during the start-up years), because by the beginning of this period, a large majority of independent puma in the population will be marked, and sampling schemes will be formalized.

Puma on the study area that may be involved in depredation of livestock or human safety incidences may be lethally controlled. Researchers that find that GPS-collared puma have killed domestic livestock will record such incidents to facilitate reimbursement to the property owner for loss of the animal(s). In addition, researchers will notify the Area Manager of the CDOW of Wildlife if they perceive that an individual puma may be a threat to public safety.

Field Methods

<u>Puma Capture</u>: Realizing that puma live at low densities and capturing puma is difficult, as a starting point, our logistical aim will be to have a *minimum* of 6 puma in each of 6 categories (36 total) radio-tagged in any year of the study if those or greater numbers are present. The 6 categories are: adult female, adult male, subadult female, subadult male, female cub, male cub. Our aim is to provide more quantitative and precise estimates of puma demographics than were achieved in earlier Colorado puma studies. This relatively large number of puma might represent the large majority of the puma population on the study area, and will provide the basic data for age- and sex-specific reproductive rates, survival rates, agent-specific mortality rates, emigration rates, and movement data pertinent to sampling designs for various projects.

Assuming that the puma population density on the study area is relatively low at the beginning of this study— about 1 adult/100 km^2 and the sex ratio is equal (Anderson et al. 1992, Logan and Sweanor 2001:167), then there might be 22 adults, 11 males and 11 females. Also assuming that the total population contains 10% subadults and 34% cubs (Logan and Sweanor 2001), then there might be 4 subadults and 13 cubs with equal sex ratios in a total population of 39 puma. If we achieve our logistical aim in the first 1—2 years (recognizing that the population might grow), then we should be able to quantify population characteristics and vital rates for a majority of the puma population in those years and build upon the tagged number in each subsequent year. Thus, our inferences will pertain to the large majority of the puma population, if not the population on the study area, instead of a relatively small sample of it. We anticipate it may take 2 years to mark the large majority of puma in the population. In addition, the study area is large and will require some time to learn to access it efficiently.

Puma capture and handling procedures have been approved by the CDOW Animal Care and Use Committee (file #08-2004). All captured puma will be examined thoroughly to ascertain sex and describe physical condition and diagnostic markings. Age of adult puma will be estimated initially by the gum-line recession method (Laundre et al. 2000) and dental characteristics of known-age puma (Logan and Sweanor, unpubl. data). Ages of subadult and cub puma will be estimated initially based on dental and physical characteristics of known-age puma (Logan and Sweanor unpubl. data). Body measurements recorded for each puma will include at a minimum: mass, pinna length, hind foot length, plantar pad dimensions. Tissue collections will include: skin biopsy (from the pinna receiving the 6 mm biopsy punch for the ear-tags) and blood (30 ml from the saphenous or cephalic veins) for genotyping individuals, parentage and relatedness analyses; disease screening; hair (from various body regions) and fecal DNA for genotyping tests of field gathered samples. Universal Transverse Mercator Grid Coordinates on each captured puma will be fixed via Global Positioning System (GPS, North American Datum 27).

Puma will be captured year-round using 4 methods: trained dogs, cage traps, foot-hold snares, and by hand (for small cubs). Capture efforts with dogs will be conducted mainly during the winter when snow facilitates thorough searches for puma tracks and the ability of dogs to follow puma scent. The study area will be searched systematically multiple times per year by four-wheel-drive trucks, all-terrain vehicles, snow-mobiles, walking, and possibly horse- or mule-back. When puma tracks ≤1 day old are detected, trained dogs will be released to pursue puma to capture.

Puma usually climb trees to take refuge from the dogs. Adult and subadult puma captured for the first time or requiring a change in telemetry collar will be immobilized with Telazol (tiletamine hydrochloride/zolazepam hydrochloride) dosed at 5 mg/kg estimated body mass (Lisa Wolfe, DVM, CDOW, attending veterinarian, pers. comm.). Immobilizing agent will be delivered into the caudal thigh muscles via a Pneu-Dart® shot from a CO_2-powered pistol. Immediately, a 3m-by-3m square nylon net will be deployed beneath the puma to catch it in case it falls from the tree. A researcher will climb the tree, fix a Y-rope to two legs of the puma and lower the cat to the ground with an attached climbing rope. Once the puma is on the ground, its head will be covered, its legs tethered, and vital signs monitored (Logan et al. 1986). (Normal signs: pulse ~70—80 bpm, respiration ~20 bpm, capillary refill time ≤2 sec., rectal temperature ~101^{o}F average, range = 95—104^{o}F) (Kreeger 1996).

A cage trap will be used to capture adults, subadults, and large cubs when puma can be lured into the trap using road-killed or puma-killed ungulates (Sweanor et al. 2005). Efficiency of the trap might be enhanced by using an automated digital call box that emits puma vocalizations (Wildlife Technologies, Manchester, NH). A cage trap will be set only if a target puma scavenges on the lure (i.e., an unmarked puma, or a puma requiring a collar change). Researchers will continuously monitor the set cage trap from about 1 km distance by using VHF beacons on the cage and door. This allows researchers to be at the cage to handle captured puma within 30 minutes. Puma will be immobilized with Telazol injected into the caudal thigh muscles with a pole syringe. Immobilized puma will be restrained and monitored as described above. If non-target animals are caught in the cage trap, we will open the door and allow the animal to leave the trap.

Foot-hold snares will be used to capture adults, subadults, and large cubs only when safe snare sites at puma kills can be located as described by Logan et al. (1999). Snares set at puma kills will be monitored continuously with VHF beacons on the snares from about 1 km distance. We will not set snares at sites where tracks indicate that other mammals (e.g., deer, elk, bear, bighorn sheep, livestock) are also active. Puma will be immobilized with Telazol injected into the caudal thigh with a pole syringe.

Vital signs will be monitored during the handling procedures. Efficiency of snares might also be enhanced with the use of an automated call box with puma or prey vocalizations.

Small cubs (≤10 weeks old) will be captured using our hands (covered with clean leather gloves) or with a capture pole. Cubs will be restrained inside new burlap bags during the handling process and will not be administered immobilizing drugs. Cubs at nurseries will be approached when mothers are away from nurseries (as determined by radio-telemetry). Cubs captured at nurseries will be removed from the nursery a distance of ~100 m to minimize disturbance and human scent at nurseries. Immediately after handling processes are complete, cubs will be returned to the exact nurseries where they were found (Logan and Sweanor 2001).

Marking, Global Positioning System, and Radio-telemetry: Puma do not possess easily identifiable natural marking, such as tigers (see Karanth and Nichols 1998, 2002), therefore, the capture, marking, and GPS- or VHF- collaring of individual puma is essential to a number of project objectives, including estimating vital rates and gathering movement data on puma to formalize designs for developing and testing enumeration methods. Adult, subadult, and cub puma will be marked 3 ways: GPS/VHF- or VHF-collar, ear-tag, and tattoo. The identification number tattooed in the pinna is permanent and cannot be lost unless the pinna is severed. A colored (bright yellow or orange), numbered rectangular (5 cm x 1.5 cm) ear-tag (Allflex USA, Inc., DFW Airport, TX) will be inserted into each pinna to facilitate individual identification during direct recaptures. Cubs ≤10 weeks old will be ear-tagged in only one pinna.

Locations of GPS- and VHF-collared puma will be fixed about once per week from light fixed-wing aircraft (e.g., Cessna 182) fitted with radio signal receiving equipment (Logan and Sweanor 2001). This monitoring will enable researchers to find GPS-collared puma to acquire remote GPS location reports from the ground, monitor the status (i.e., live or dead) of individual puma, and to recover carcasses for necropsy. It will also provide simultaneous location data on mothers and cubs. GPS- and VHF-collared puma will be located from the ground opportunistically using hand-held yagi antenna. At least 3 bearings on peak aural signals will be mapped to fix locations and estimate location error around locations (Logan and Sweanor 2001). Aerial and ground locations will be plotted on 7.5 minute USGS maps (NAD 27) and UTMs along with location attributes will be recorded on standard forms. GPS locations will be mapped using ArcGIS software.

Adult and subadult female puma will be fitted with GPS collars (approximately 400 g each, Lotek Wireless, Canada). Initially, GPS-collars will be programmed to fix and store puma locations at 4 times per day to sample daytime, nighttime, and crepuscular locations (i.e., 0:00, 06:00, 12:00, 19:00). GPS locations for puma will provide precise, quantitative data on puma movements mainly to provide data to formalize study designs, to test assumptions for capture-mark-recapture methods for this project, and to assess the relevance of puma DAU boundaries. The GPS-collars also will provide basic information on puma movements and locations to design other pilot studies in this program on vulnerability of puma to sport-harvest, habitat use, and predation frequency on mule deer and elk.

Subadult male puma will be fitted initially with conventional VHF collars (Lotek, LMRT-3, ~400 g each) with expansion joints fastened to the collars, which allows the collar to expand to the average adult male neck circumference (~46 cm). If subadult male puma reach adulthood on the study area, we will recapture them and fit them with GPS collars.

VHF radio transmitters on GPS collars will enable researchers to find those puma on the ground in real time to acquire remote GPS data reports, facilitate recaptures for re-collaring, and to check on their reproductive and physical status. VHF transmitters on GPS- and VHF-collars will have a mortality mode set to alert researchers when puma have been immobile for at least 3 hours so that dead puma can be found to quantify survival rates and agent-specific mortality rates by gender and age.

We will attempt to collar all cubs in observed litters with small VHF transmitter mounted on an expandable collar (~100g, MOD 210, Telonics, Inc., Mesa, Arizona) when cubs weigh 2.3—11 kg (5—25 lb). Cubs with mass ≥11 kg can still wear these small expandable collars until they are about 12 months old. Cubs approaching the age of independence (~11—14 mo. old) may be fit with Lotek LMRT-3 VHF collars (~400 g) with expansion links. Cubs will be recaptured to replace collars as necessary. Monitoring radioed cubs allow quantification of survival rates and agent-specific mortality rates (Logan and Sweanor 2001).

Capture-Mark-Recapture: Capture-mark-recapture methods will be evaluated initially as a pilot study. Capturing and marking puma is time consuming, and would lengthen the time to thoroughly search the study area for capturing and marking puma during capture-recapture occasions needed for population estimation. Therefore, we will capture and mark puma prior to performing capture-recapture occasions using houndsmen teams. In addition, by marking puma before capture-recapture occasions begin, we will have opportunities to capture female puma at different stages of their reproductive status, and thus reduce the chance that mothers in a stage with suckling cubs and small activity areas are not detected and marked on the study area. After cubs are weaned, the mothers' activity area expands (Logan and Sweanor 2001). The probability of females having suckling cubs in winter is naturally small; that season exhibits the lowest rate of births (Logan and Sweanor 2001). Capture-recapture occasions to estimate the population of independent puma may not begin until the end of the second winter or the third winter when we have a large majority of the puma population sampled and marked. Occasions performed at that time will be viewed as a pilot study allowing us to examine the logistics of the field methods, the extent to which model assumptions are met, performance of field methods (e.g., detection differences by sex or life stage as revealed by GPS data on collared puma), and precision of capture-recapture models used to estimate the puma population.

Analytical Methods

Population Characteristics: Population characteristics each year will be tabulated with the number of individuals in each sex and age category. Age categories, as mentioned, include: adult (puma ≥24 months old, or younger breeders), subadults (young puma independent of mothers, <24 months old that do not breed), cubs (young dependent on mothers, also known as kittens) (Logan and Sweanor 2001). When data allow, age categories may be further partitioned into months (for cubs and subadults) or years (for adults).

Reproductive Rates: Reproductive rates will be estimated for GPS- and VHF-collared female puma directly (Logan and Sweanor 2001). Genetic paternity analysis will be used to ascertain paternity for adult male puma (Murphy et al. 1998). Methods will be tested in Dr. M. Douglas's Laboratory (Colorado State University, Department of Fishery and Wildlife Biology).

Survival and Agent-specific Mortality Rates: Radio-collared puma will provide known fate data which can be used to analyze survival rates in program MARK (White and Burnham 1999, Cooch and White 2004). Agent-specific mortality rates will be analyzed using proportions and Trent and Rongstad procedures (Micromort software, Heisey and Fuller 1985). Cub survival curves for each gender will be plotted with survival rate on age in months (Logan and Sweanor 2001:119).

Population Estimates: Capture-recapture models will be evaluated initially as a pilot study to estimate the parameters of primary interest— absolute numbers of independent puma (i.e., number of adult and subadult puma present in the survey area) and puma density (i.e., number of independent puma/100 km^2) each winter— December through March— when snow facilitates detection and capture of puma, provided that we meet model assumptions. The December―March period also corresponds with Colorado's puma hunting season. The population of interest is independent puma (i.e., adults and subadults) because those are the puma that can be legally killed by recreational hunters. Furthermore, adults comprise the breeding segment of the population and subadults are non-breeders that are potential recruits into the adult population in ≤1 year. Thus, the sampling unit is the individual independent puma (~≥1 yr. old).

General assumptions for closed capture-recapture models are: (1) the population is closed; (2) animals do not lose their marks during the interval; (3) all marks are correctly noted and recorded at each trapping occasion; (4) each animal has a constant and equal probability of capture on each capture occasion. Open population models allow the assumption of closure to be relaxed (Otis et al. 1978, White et al. 1982, Pollock et al. 1990). The robust design is a combination of closed and open models; thus, assumptions are a combination of the assumptions for closed and open population methods (Kendall 2001).

To analyze capture-recapture data, closed, open, and the robust design models are available in program MARK. Akaike's Information Criterion will be used to select the most parsimonious models based on AICc score ranks and the difference in AIC (ΔAIC) between models (Burnham and Anderson 1998). MARK results also include estimates of abundance.

Because the precision of estimates for small populations is sensitive to the probability of capture (White et al. 1982, Pollock et al. 1990), our operational goal will be to achieve capture probabilities of at least 0.5 for each animal per capture occasion. Capture simulations using MARK software (Cooch and White 2004) indicate that greater capture probabilities and more capture occasions yield more precise estimates. The capture probability for the simplest closed model [M(o)], which assumes that every member of the population has the same probability of capture *(p)* for each sampling period, suggest that for a population of 30 animals (i.e., adults plus subadult puma, which might be present by the end of year 2, see Puma Capture above) *p* must equal 0.5 for 3 capture occasions to attain a coefficient of variation (*V)* of 0.1. If 6 capture occasions are used, then a *p* of 0.3 might yield a *V* of 0.09.

In addition, behavior, movements, survival and mortality of GPS- and VHF-collared puma will allow direct biological examinations of assumptions of geographic and demographic closure (White et al. 1982) and variation in capture probability of individual puma and puma classes (i.e., adult females, adult males, subadult females, subadult males). If capture probabilities vary by puma class, we will examine if data stratification is necessary or possible (depending upon sample size). For example, we might expect the larger home ranges of male puma to expose them to more search routes, thus, this may increase their probability of capture. If the assumption of demographic closure cannot be satisfied, then open population models and the robust design would be more appropriate (Pollock et al. 1990, Williams et al. 2001). Collared puma will allow us to determine the number of marked puma present in the search area each capture-recapture occasion. Furthermore, GPS locations (4 fixes/day) on individual puma will provide data on the probability that puma may temporarily move out of and back into the survey area between capture occasions. Unmarked puma that are subsequently GPS-collared should provide such information, too.

ArcView geographic information system software will be used to map and analyze puma locations, movements, and home ranges. It will also be used to map and quantify attributes of the study area and sampling frames.

Rate of Population Increase: Finite rates of increase ($\lambda = N_{t+1}/N_t$) between consecutive years and average annual rates of increase *(r)* for 3- to 5-year periods and levels of precision will be calculated (Caughley 1978, Van Ballenberghe 1983) and plotted.

Functional Relationships: Graphical methods will be used to examine functional relationships between puma density and vital rates, relationships between puma density estimated with direct capture-recapture methods (i.e., houndsmen teams) and possibly later (depending upon funding) by using estimates from DNA genotype mark- recapture methods. Linear regression procedures and coefficients of determination can be used to assess these functional relationships if data for the response variable are normally distributed and the variance is the same at each level. If the relationship is not linear, data is non-normal, and variances are unequal, we will consider appropriate transformations of the data for regression procedures (Ott 1993). Non-parametric correlation methods, such as Spearman's rank correlation coefficient, can also be used to test for monotonic relationships between puma abundance and other parameters of interest (Conover 1999).

Statistical analyses will be performed using SYSTAT and SAS software. The risk of committing a type I error (i.e., rejecting a null hypothesis that is actually true) will be controlled at alpha = 0.10 because we will normally have small population sizes (typical of studies of large obligate carnivores). The higher alpha level will increase the probability of detecting a change and reduce the risk of a type II error (i.e., failing to reject a null hypothesis that is false). For managers, the risk of a type II error is probably more important.

RESULTS AND DISCUSSION

Segment Objective 1

The Division of Wildlife held public meetings in Redvale (August 23, 2004) and Montrose (August 30, 2004) where DOW staff informed attendees from the Uncompahgre Plateau area about the puma research project and addressed their questions. Meetings were held with over 70 private landowners, ranchers, hunters, outfitters, and guides that live and operate on the Uncompahgre Plateau to inform them about the puma research, address questions, and request permission to access private lands for puma research activities. Additional meetings were with representatives of the U.S. Forest Service, Bureau of Land Management, National Park Service, and Colorado State Parks who were also informed about the puma research.

Segment Objective 2

The Wildlife Commission passed regulations allowing for the experimental design of this puma research. Their decision resulted in a closure to puma sport-hunting for the first 5-years of the research (Nov. 11, 2004 to Mar. 31, 2009) on the study area. In addition, the Commission allowed the creation of a buffer zone during the same time period comprised of the remaining parts of Game Management Units 61 and 62 north of the 25 Mesa Road (i.e., north of the study area) where pumas tagged on the study area can not be legally taken by puma sport-hunters. The buffer zone is intended to protect puma that are originally captured and sampled in the study area and that range to the north so that pumas in the study population will express life history traits not affected by sport-hunting off-take. A larger buffer zone to protect pumas tagged on the study area was requested of the CDOW Regulations Review Committee. That buffer zone would have protected all puma tagged in the study area even if they ranged off the study area but were west of the continental divide in Colorado. However, that request was denied by the Regulations Review Committee.

Segment Objective 3

A study plan was developed, peer-reviewed, modified with the peers' recommendations (Logan 2004), and then initiated to begin the long-term, experimental research on puma population dynamics on the Uncompahgre Plateau. Procedures for the capture, restraint, handling, and sampling of pumas for this research were reviewed and approved (file #08-2004) by the Colorado Division of Wildlife Animal Care and Use Committee.

Segment Objectives 4—7

Field research to begin quantifying puma population structure, vital rates, and causes of mortality began on Dec. 2, 2004. From December 2, 2004 to May 12, 2005, trained dogs were used as our main method to capture, sample, and mark pumas. Our search efforts on the east slope of the study area were from 25 Mesa Road south to Fisher Creek. On the west slope, our efforts were from the 25 Mesa Road south to Goodenough Gulch. Those efforts resulted in 78 search days, 109 puma tracks, 35 pursuits, and the capture of 14 pumas (Table 1). Eight of those pumas were restrained, sampled, tagged, and released (Table 2). Puma M1 was unintentionally recaptured when we thought we were pursuing an unmarked puma. Puma F3 was recaptured during our effort to capture her male offspring M5 for the first time.

Pumas were bayed in trees by dogs on 4 other occasions, but we did not attempt to anesthetize the puma because of concern for the pumas safety on 3 occasions, and concern for the puma's and the researchers' safety on one occasion (Table 3). In those cases, a puma was treed in cliffs at night, and on 3 occasions the pumas were bayed in trees too dangerous for researchers to attempt to safely dart the pumas and then climb the trees to retrieve the cats. These pumas included 1 large adult female that was probably caught twice, 1 adult male, and 1 puma that was either a large cub or a subadult (sex undetermined). A summary of capture efforts with dogs is in Table 4.

We attempted to capture a female puma on Ridgeway State Park on April 1, 2005. The puma killed an adult mule deer doe, and had begun to eat the deer on the sidewalk beside the Fishing Pond at Pa-Co-Chu-Puk Campground. This location was about 520 m east of where our trained dogs treed, but we could not handle a large female puma on February 1, 2005. We used a cage trap designed for black bears to attempt to capture the puma, but the bear trap was not sufficient. The puma entered the trap, but apparently the cage door did not latch because the puma's tail was caught in the door jam. The puma did not return to cage trap. We did not pursue the puma with dogs because of the close proximity of highway 550 and private lands directly north and east of the park.

We captured 8 cubs from 4 litters born to GPS-collared female pumas. Two litters were born in May, 1 litter was born in June, and 1 litter was born in August. There were 3 males and 5 females (Table 5).

One puma death was detected on July 28, 2005. A female, about 49 months old, was hit and killed by a car between 06:00—08:00 on state highway 62 about 10.4 km west of Ridgeway in lower Cottonwood Creek. This location was on the southern boundary of the study area. A necropsy showed that the puma appeared to be in excellent physical condition prior to its death. Her mass was 46 kg; she apparently was not pregnant; and her mammary glands were not producing milk.

Segment Objectives 8—9

Seven adult pumas were fit with Lotek 4400S GPS collars programmed to fix 4 locations per day (00:00, 06:00, 12:00, and 19:00). The number of GPS locations per individual puma ranged from 355 to 779 (Table 6). Because none of the puma have yet been monitored for a complete year and the sample is small, annual and seasonal home ranges sizes were not estimated for this report. However, we estimated the activity areas used by the 7 GPS-collared adult pumas (Table 6) during the monitoring periods and overlaid 100% Minimum Convex Polygons on a map of the study area (Fig. 2). In addition, we are collaborating with colleagues at Colorado State University— Dr. K. Crooks, Dr. D. Theobald, and Dr. K. Wilson— to develop a proposal and funding that would allow us to develop and validate puma habitat suitability models and maps for Colorado in which these puma GPS location data will be used.

Tissue samples from all of the captured pumas and the unmarked female puma hit and killed by a car have been archived with geneticist Dr. M. Douglas. We are currently collaborating with Dr. Douglas to develop a study plan and funding for the development and assessment of laboratory and field methods for genotyping pumas and for estimating puma abundance in the wild using DNA mark-recapture techniques.

We conducted a preliminary assessment of the usefulness of GPS-collar technology for investigations of puma-prey relationships. The average GPS location fix rate for the 7 GPS-collared pumas was 70.7% (range = 54—87%) (Table 6). We investigated 139 GPS location clusters for 7 adult pumas where individual GPS-collared pumas spent ≥1 day during the span December 26, 2004 to July 31, 2005. The estimated error between 101 collar-fixed GPS locations and prey remains found on the ground averaged 3.2 m (range = 0—50 m, *SE* = 0.6). Prey remains were found at 112 of the 139 clusters, with mule deer and elk comprising 54% and 43%, respectively (Table 7). The sex and age stage structure of 60 mule deer and 48 elk used by puma at GPS clusters is in Table 8. On average, puma spent 2.3 days on mule deer (range = 1—6, *SE* = 0.2) and 2.9 days on elk (range = 1—10, *SE* = 0.3). Ungulate use rates by the GPS-collared pumas estimated from these data are in Table 9. Evidence that black bears (*Ursus Americana*) used portions of the same ungulates used by GPS-collared pumas was found at remains of 7 mule deer and 10 elk. Evidence that coyotes (*Canis latrans*) used portions of the same ungulates used by GPS-collared pumas was found at remains of 7 mule deer and 14 elk. We are currently assessing how this GPS-collar capability could be used to structure research on puma-ungulate relationships on the Uncompahgre Plateau and the additional funding and personnel needed to thoroughly execute the research.

SUMMARY

Experimental, long-term research on puma population dynamics, effects of sport-hunting, and development and testing of puma enumeration methods began in December 2004. From December 2004 to July 2005 fifteen pumas were captured, sampled, marked, and released. The number of pumas handled is partially contingent upon effort, the number of pumas present on the study area, and safety concerns. Individual pumas sampled in the population provide quantitative information on population structure, vital rates, and dynamics over time in *reference* and *treatment* periods to improve the CDOW's puma management. All pumas were sampled as part of developing research for genotype mark-recapture procedures. Seven adult puma were fit with GPS collars, yielding 487—779 locations. Puma GPS location data will be used to: design enumeration methods in the field, develop and test puma habitat suitability models and maps, and develop potential research on puma-ungulate relationships on the Uncompahgre Plateau contingent upon funding and support.

Research efforts for year 2 will focus on increasing numbers and distribution of sampled, marked, and GPS/radio-collared puma on the study area for data to address the objectives, management assumptions, and hypotheses in the study plan. We will further develop proposals for the puma genetics research, puma habitat suitability models and maps, and puma-prey relationships.

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Table 1. Puma capture efforts with dogs from December 2, 2004 to May 12, 2005, Uncompahgre Plateau, Colorado.

Month	No. Search Days	No. & type of puma tracks found[a]	No. & type of pumas pursued	No. & I.D. or type of pumas captured
December	19	20 tracks: 11 male, 9 female	4 pursuits: 3 males, 1 female	2 pumas captured: M1, 1 female not handled
January	15	26 tracks: 9 male, 15 female, 2 cub	8 pursuits: 4 males, 4 females	4 pumas captured: M1 recaptured, F2, F3, M4
February	17	22-23 tracks: 5 male, 14 female, 2-4 cubs, or 2-3 cubs & 1 subadult	11 pursuits: 2 males, 7 females, 2 cubs, or 1 cub & 1 subadult	6 pumas captured: 1 female not handled, F3 recaptured, cub M5, M6, 1 cub or subadult, F7
March	11	17 tracks: 8-9 male or 1 large cub, 7 female, 1 unspecified sex	2 pursuits: 2 females	1 puma captured: F8
April	9	13 tracks: 10 male, 3 female	2 pursuits: 2 males	1 puma captured: 1 male not handled
May	7	10 tracks: 4 male, 6 female	8 pursuits: 3 males, 5 females	0 pumas captured
TOTALS	78	109 tracks found: 47-48 male, 54 female, 4-6 cub or 0-1 subadult, 1 unspecified	35 pursuits: 14 males, 19 females, 1 male cub, 1 cub (unknown sex) or 1 subadult (unknown sex)	14 captures: (6 males, 6 females, 1 male cub, 1 cub (unknown sex) or 1 subadult (unknown sex)

[a] Puma hind-foot tracks with plantar pad widths >52 mm wide are assumed to be male; ≤52 mm are assumed to be female.

Table 2. Pumas that were captured with aid of dogs, sampled, tagged, and released from December 2, 2004 to May 12, 2005, Uncompahgre Plateau, Colorado.

Puma I.D.	Sex	Estimated Age (mo.)	Mass (kg)	Capture date	Location
M1	male	33	68	12-08-04	Shavano Valley
M4	male	25-33	65	01-28-05	McKenzie Butte Mesa
M5	male	6	12	02-04-05	Spring Creek
M6	male	33	59	02-18-05	Happy Canyon
F2	female	49	43	01-07-05	Dolores Canyon
F3	female	41	40	01-21-05	Spring Creek
F7	female	56-64	32	02-24-05	Dolores Canyon
F8	female	21	30	03-21-05	Cottonwood Creek (W)

Table 3. Pumas that were captured with aid of dogs, but were not handled for safety reasons, from December 2, 2004 to May 12, 2005, Uncompahgre Plateau, Colorado.

Puma sex	Age stage	Capture date	Location	Comments
Female	adult	12-23-05	McKenzie Butte Mesa	Large female.
Female	adult	02-01-05	South McKenzie Butte Mesa	This puma probably same animal caught 12-23-05.
Unspecified	cub or subadult	02-24-05	Dolores Canyon	This puma apparently in association with F7 at an elk kill. Possibly F7's offspring or an unrelated subadult.
Male	adult	04-05-05	Horsefly Canyon (E)	This puma, or another male, was pursued on 4 other occasions in the San Miguel River-to-Cottonwood Creek area.

Table 4. Summary of puma capture efforts with dogs, December 2004 to May 2005, Uncompahgre Plateau, Colorado.

Period	Track detection effort	Pursuit effort	Puma capture effort	Effort to capture a puma for the first time
Dec. 2, 2004 to May 12, 2005	109/78 = 1.40 tracks/day	35/78 = 0.45 pursuit/day 78/35 = 2.23 day/pursuit	14/78 = 0.18 capture/day 78/14 = 5.57 day/capture	11 pumas captured for first time (minus M1, F3, & large female) 11/78 = 0.14 capture/day 78/11 = 7.09 day/capture

Table 5. Puma cubs sampled on the Uncompahgre Plateau Puma Study area, 2004 to 2005.

Cub I.D.	Sex	Estimated birth date	Estimated age at capture	Mass (kg)	Mother	Estimated age of mother at birth of this litter (mo)
M5	male	August 2004[a]	6 months	12	F3	36
F9	female	May 28, 2005[b]	31 days	2.27	F2	44
F10	female	May 28, 2005[b]	31 days	2.04	"	"
M11	male	May 28, 2005[b]	31 days	2.27	"	"
F12	female	May 19, 2005[b]	42 days	2.63	F7	59-67
F13	female	May 19, 2005[b]	42 days	1.72	"	"
F14	female	June 26, 2005[b]	26 days	1.90	F8	24
M15	male	June 26, 2005[b]	26 days	2.0	"	"

[a] Estimated age of M5 was based on morphometric comparisons with known-age cubs (Logan and Sweanor 2001, and unpublished data).

[b] Estimated age of cubs sampled at nurseries is based on the starting date for GPS location foci for mothers at nurseries.

Table 6. Numbers of GPS locations for adult puma on the Uncompahgre Plateau, Colorado, December 2004 to August 2005.

Puma I.D.	Sex	Age stage	Dates monitored [a]	No. locations	Acquisition rate average, range, *n*[b]	Use areas estimated (km^2) with 100% Minimum Convex Polygon[c]
M1	male	adult	12-08-04 to 08-19-05	779	76, 73—83, 5	815
M4	male	adult	01-28-05 to 07-25-05	487	73, 57—84, 5	254
M6	male	adult	02-18-05 to 07-25-05	543	87, 82—93, 5	552
F2	female	adult	01-07-05 to 08-10-05	565	65, 43—82, 7	120
F3	female	adult	01-21-05 to 08-02-05	586	76, 67—85, 6	174
F7	female	adult	02-24-05 to 07-26-05	362	54, 26—78, 5	94
F8	female	adult	03-21-05 to 08-08-05	355	64, 48—78, 4	245

[a] GPS collars on pumas are remotely downloaded at approximately 1-month intervals. The last date in *Dates monitored* is for the last location from the last GPS data download for an individual puma for this report.

[b] *n* = number of remote downloads.

[c] Polygons for individual GPS-collared puma are overlaid on a study area map in Figure 2.

Table 7. Observations at GPS location clusters for 7 GPS-collared puma on the Uncompahgre Plateau, Colorado, December 2004 to July 2005.

Puma I.D.	No. GPS clusters	Dates of GPS clusters that were investigated	Mule deer	Elk	Porcupine	Beaver	Puma scavenge or share[a]	Only Puma sign[b]	Only Black bear sign[c]	Nothing found	No. GPS clusters not visited[d]
M1	23	12-26-04 to 07-10-05	4	14		1				4	2
M4	16	02-03-05 to 07-12-05	4	7				1		4	2
M6	17	02-18-05 to 07-07-05	3	11			2	1		0	4
F2	26	01-12-05 to 07-26-05	12	9			2	2	1	0	1
F3	27	01-27-05 to 07-31-05	22	5						0	0
F7	18	03-08-05 to 07-22-05	9		1			5	1	2	0
F8	11	03-23-05 to 07-03-05	7	2	1			1		1	0
Total	139		61	48	2	1	4	10	2	11	9

[a] A GPS-collared puma either shared a prey item with another GPS-collared puma (2 instances), or a GPS-collared puma scavenged on remains of prey previously used by another GPS-collared puma (2 instances).

[b] Only puma tracks, feces, and/or beds were found at the GPS cluster.

[c] Only black bear sign (e.g., feces) was found at the puma GPS cluster.

[d] Some puma GPS clusters were not investigated because clusters fell on small private land holdings where we did not have permission for access at the time, or other principal objectives of our research were priority.

Table 8. Sex and age structure of mule deer and elk found at GPS location clusters for 7 GPS-collared adult puma on the Uncompahgre Plateau, Colorado, December 2004 to August 2005.

	Sex	No.	Fawn/Calf	Yearling	2+ years	Unknown age
Mule deer	Female	26	2	2	20	2
	Male	10	0	3	7	0
	Unknown	25	13	3	3	6
	Total	61	15	8	29	8
Elk	Female	25	12	1	12	0
	Male	5	0	0	5	0
	Unknown	18	16	0	1	1
	Total	48	28	1	18	1

Table 9. Estimated ungulate use rates of adult GPS-collared pumas on the Uncompahgre Plateau, Colorado, December 2004 to July 2005.

Puma I.D.	Dates starting with & ending with ungulate use	No. days inclusive in date span	No. ungulates used	Estimated No. ungulates used per year[a]
M1	12-26-04 to 07-10-05	196	18	33.5
M4	02-03-05 to 07-04-05	152	11	26.4
M6	02-18-05 to 07-07-05	140	14	36.5
F2	01-12-05 to 07-26-05	195	21	39.3
F3	01-27-05 to 07-31-05	185	27	53.3
F7	03-08-05 to 07-16-05	131	9	25.1
F8	03-23-05 to 07-03-05	103	9	31.9

[a] Estimated ungulate use rates per year are based on the key assumption that the individual puma would use ungulates throughout the year equal to the same rate recorded during the monitoring span in *Dates starting with & ending with ungulate use*. This assumption is probably not reliable especially for adult female pumas, because their reproductive status, and thus energetic needs vary throughout the year. For example, F3 was raising cubs born in August 2004; yet, F2, F7, and F8 started raising cubs born in May, May, and June of 2005, respectively. In addition, not all GPS clusters were investigated for M1, M4, M6, and F2 (see Table 7).

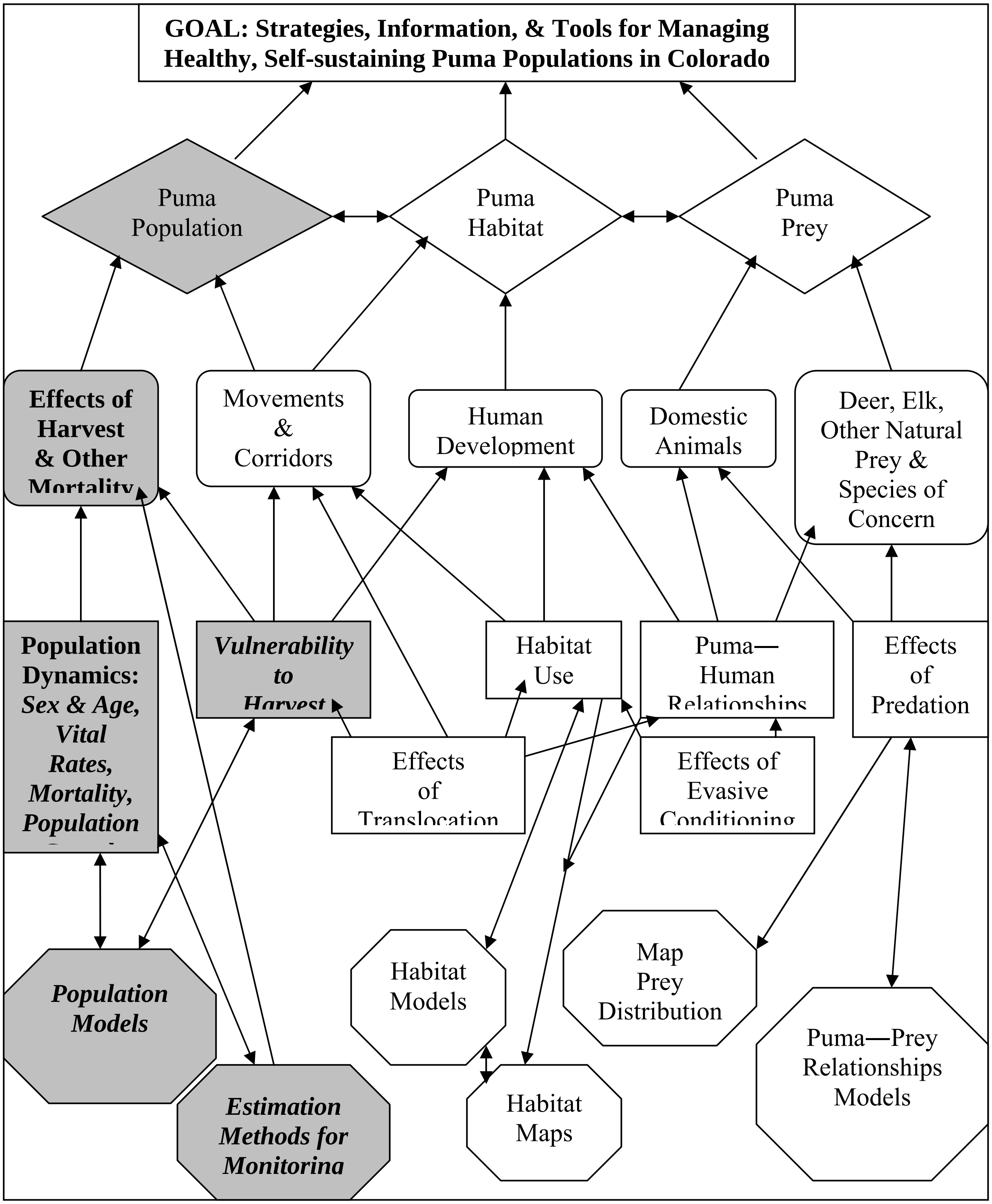

Figure. 1. An ecologically-based conceptual model of the Colorado Puma Research Program that provides the contextual framework for this and proposed puma research in Colorado. Gray-shaded shapes identify areas of research addressed by this report and the puma management goal (at top).

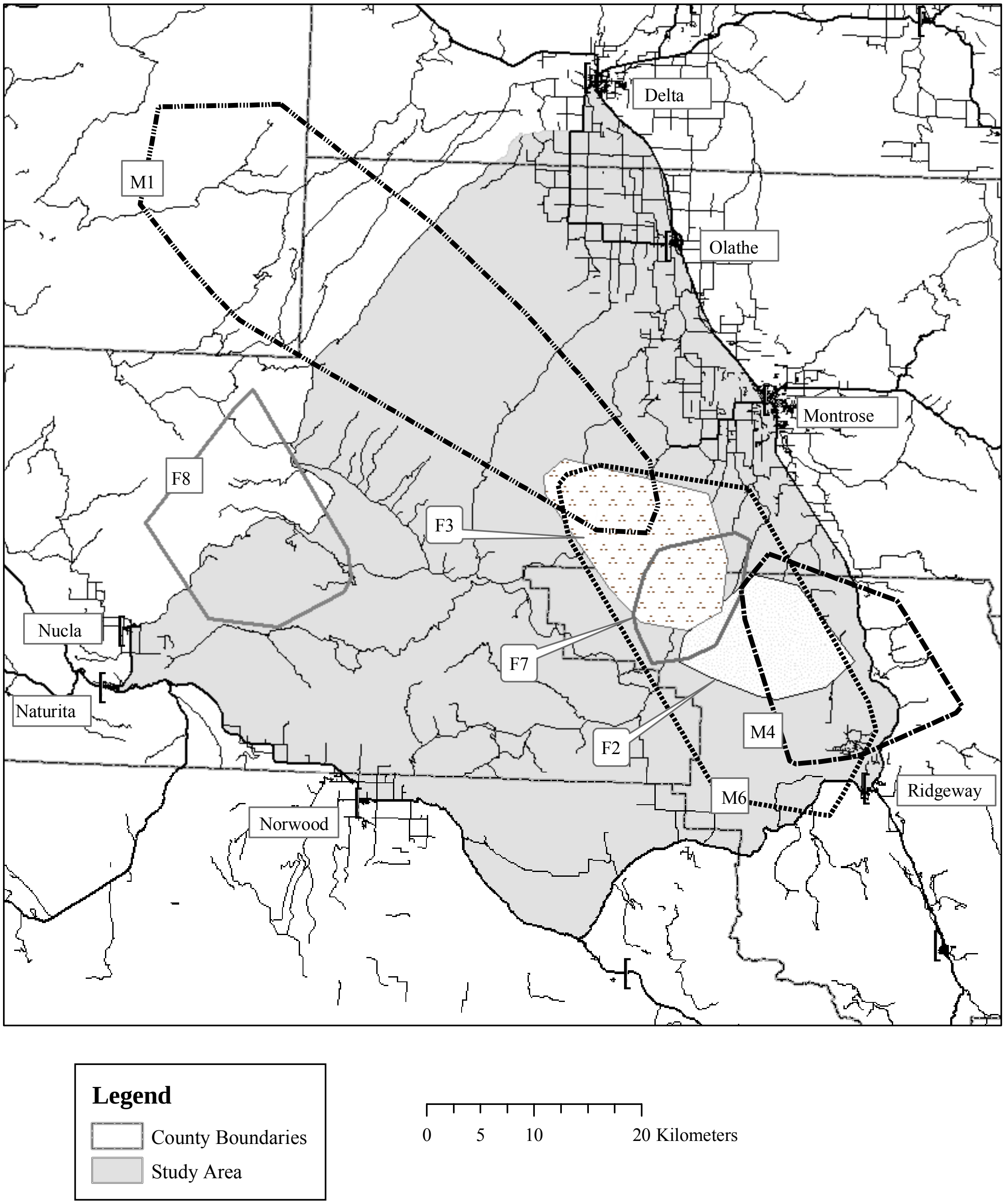

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Figure 2. The Uncompahgre Plateau Puma Study Area with activity areas of adult GPS-collared pumas depicted with 100% Minimum Convex Polygons, December 2004 to August 2005.

Colorado Division of Wildlife
July 2004 - June 2005

WILDLIFE RESEARCH REPORT

State of	Colorado	: Division of Wildlife
Cost Center	3430	: Mammals Research
Work Package No.	7210	: Customer Support Services/Research Support
Task No.	1	: Library Services

Federal Aid Project: N/A :

Period Covered: July 1, 2004 – June 30, 2005

Author: J. A. Boss

Personnel: J. A. Boss

All information in this report is preliminary and subject to further evaluation. Information MAY NOT BE PUBLISHED OR QUOTED without permission of the author. Manipulation of these data beyond that contained in this report is discouraged.

ABSTRACT

During the Segment, the following were accomplished:

722 Publications acquired by the Research Center Library for the use of Colorado Division of Wildlife (CDOW) employees, cooperators, wildlife educators, and the public. These publications include books, interlibrary loan materials, periodicals, and newsletters.

1,593 Items of information delivered to CDOW employees, cooperators, wildlife educators, and the public, resulting from requests and literature searches.

469 Items of information cataloged into the electronic and card catalogues, which including duplicates and additional volumes expanded the Research Center Library inventory to 24,293 items.

703 Items of information entered into the electronic catalog for the maintenance of the materials collection of the Research Center Library.

1,322 Items checked-out by CDOW employees, cooperators, wildlife educators, and the public indicating use of library services.

2,251 Items of information delivered that are produced by the CDOW employees, cooperators, wildlife educators, and the public. These items include CDOW and other publications (1,552) research articles by CDOW personnel (464), and CDOW Wildlife Research Reports (235).

WILDLIFE RESEARCH REPORT

COLORADO DIVISION OF WILDLIFE RESEARCH LIBRARY SERVICES

JACQUELINE A. BOSS

P.N. OBJECTIVE

Provide an effective support program of library services at minimal cost through centralization and enhancement of accountability for Colorado Division of Wildlife (CDOW) employees, cooperators, wildlife educators, and the public.

SEGMENT OBJECTIVES

1. Continue to improve and modernize library services by implementing the Dynix Horizon library automation system via an Application Service Provider (ASP) model (project began in June 2002). By joining the Automation System Colorado Consortium (ASCC) we were able to take advantage of a LSTA grant written by the Colorado State Library staff, which facilitated the implementation of this system.

2. Continue to develop, improve, and implement the CDOW Research Center Library web-site (started in June 2004) by implementing the Dynix Horizon system online to serve a broader spectrum of patrons of the CDOW Research Center Library.

3. Continue to attended ASCC meetings and participate Dynix Horizon online classes to enhance utilization of the Dynix system.

SUMMARY OF LIBRARY SERVICES

Maintain and Build Electronic Catalogs of all Research Library Holdings

469	Total number of items cataloged during this period of time. This includes not only new acquisitions, but also older materials from the library collection being entered into the electronic catalog for the first time. Among the new acquisitions are Federal Aid : Job Progress Reports and manuscripts written by CDOW researchers and other employees.
703	Total number of items of information added to the electronic circulation system during this period. This includes not only the above mentioned newly cataloged items, but also newly acquired serials, volumes, additional copies, and other items being assigned scanning numbers for the electronic circulation system for the first time.
$227,820	Estimated value of the 24,293 items in the Research Center Library collection as of June 30, 2005. The project to determine the value of the library collection began in May 2000. As time permits, the value of books already in the collection is determined, and added to the already “estimated value.” Each month’s addition of values of older materials, plus the new materials, increases the value of the Library collection. Not included in the “assumed value” of the Library collection are all of the periodicals, older materials, and government documents, which continue to be a large part of the collection, thus the “estimated value” of the Library collection continues to grow month by month.

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