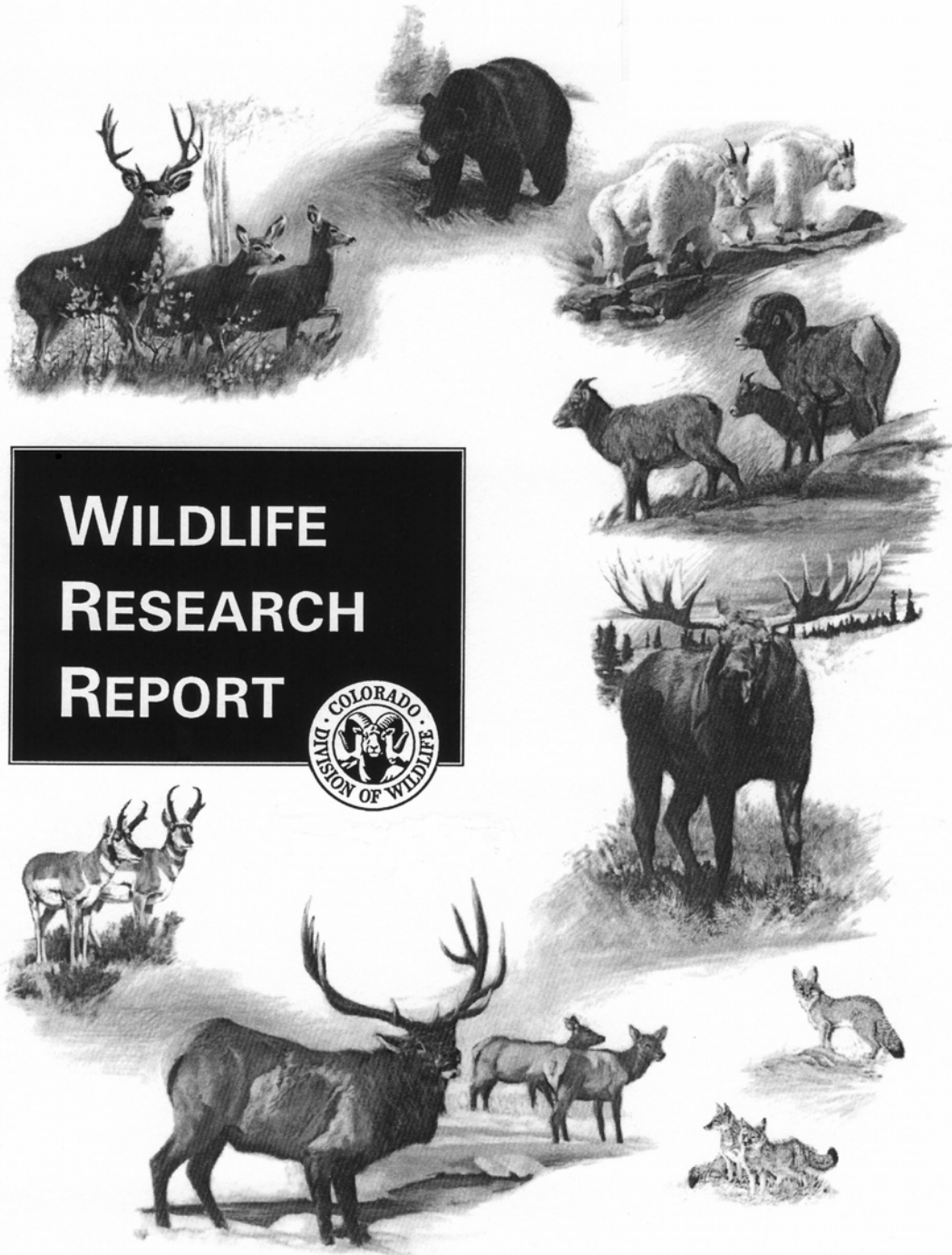


# MAMMALS - JULY 2004



## WILDLIFE RESEARCH REPORT





# **WILDLIFE RESEARCH REPORT**

**JULY 2003 – JUNE 2004**



**MAMMALS PROGRAM**

**COLORADO DIVISION OF WILDLIFE**  
Research Center, 317 W. Prospect, Fort Collins, CO 80526

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**TABLE OF CONTENTS**  
**MAMMALS RESEARCH PROGRESS REPORTS**

**PREBLE’S MEADOW JUMPING MOUSE CONSERVATION**

WP 0662 EFFECTS OF RESOURCE ADDITION ON PREBLE’S MEADOW JUMPING  
MOUSE (*Zapus hudsonius preblei*) MOVEMENT PATTERNS by T. Shenk.....1

**LYNX CONSERVATION**

WP 0670 POST-RELEASE MONITORING OF LYNX REINTRODUCED TO COLORADO  
by T. Shenk.....5

WP 0670 ECOLOGY OF SNOWSHOE HARES IN COLORADO by J. Zahratkal.....15

**BLACK-FOOTED FERRET**

WP 0880 DISEASE MONITORING AND MANAGEMENT by L. Wolfe.....17

**DEER CONSERVATION**

WP 3001 EFFECT OF NUTRITION AND HABITAT ENHANCEMENTS ON MULE  
DEER RECRUITMENT AND SURVIVAL RATES by C. Bishop.....21

**ELK CONSERVATION**

WP 3002 TECHNICAL SUPPORT FOR ELK AND VEGETATION MANAGEMENT  
ENIRONMENTAL IMPACT STATEMENT FOR ROCKY MOUNTAIN  
NATIONAL PARK by D. Baker.....45

WP 3002 ESTIMATING CALF AND ADULT SURVIVAL RATES AND  
PREGNANCY RATES OF GUNNISON BASIN ELK by D. Freddy.....57

**PUMA CONSERVATION**

WP 3003 COLORADO PUMA RESEARCH AND MANAGEMENT PROGRAM  
by K. Logan.....61

**OTHER UNGULATES CONSERVATION**

WP 3004 POTENTIAL RESEARCH PROJECT ASSESSMENT by E. Bergman.....89

**WILDLIFE DISEASES**

WP 3740 CHRONIC WASTING DISEASE IN MULE DEER – RESEARCH AND DEVELOPMENT by M. Miller and L. Wolfe.....103

WP 3740 WILDLIFE DISEASE SURVEILLANCE TECHNICAL AND LABORATORY SUPPORT by L. Baeten.....113

WP 3740 PILOT EVALUATION OF GPS TECHNOLOGY IN CHRONIC WASTING DISEASE PREVALENCE AND MANAGEMENT AT ARTIFICIAL FEEDING SITES IN URBAN AREAS by E. Bergman, M. Miller, and L. Wolfe.....119

WP 3740 VETERINARY SERVICES – MEDICAL SUPPORT by L. L. Wolfe.....123

WP 3740 ANIMAL AND PEN SUPPORT FACILITIES FOR MAMMALS RESEARCH by T. Davis.....139

**MULTISPECIES INVESTIGATIONS**

WP 3001 CONSULTING SERVICES FOR JOB MARK-RECAPTURE ANALYSIS by G. White.....151

**RESEARCH SUPPORT / ADMINISTRATIVE SERVICES**

WP 7210 LIBRARY SERVICES by J. Boss.....163

Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. \_\_\_\_\_ : Mammals Research  
Work Package No. 0662 : Preble's Meadow Jumping Mouse Conservation  
Effects of Resource Addition on Preble's  
Meadow Jumping Mouse (*Zapus hudsonius*  
Task No. 2 : preblei) Movement Patterns  
Federal Aid Project: N/A :

Period Covered: July 1, 2003 - June 30, 2004

Author: Anne M. Trainor.

Personnel: T. M. Shenk, K. R. Wilson, G. C. White

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### ABSTRACT

A thesis, entitled 'Influence of resource supplementation on movements of Preble's meadow jumping mouse (*Zapus hudsonius preblei*) and habitat use characteristics,' was completed and submitted to Colorado State University in partial fulfillment of a Master of Science degree. The thesis is available from The Colorado Division of Wildlife Library or the Colorado State University Library. Included in this report is an abstract of the thesis.

**JOB PROGRESS REPORT**  
**INFLUENCE OF RESOURCE SUPPLEMENTATION ON MOVEMENTS OF PREBLE'S**  
**MEADOW JUMPING MOUSE (*Zapus hudsonius preblei*) AND HABITAT USE**  
**CHARACTERISTICS**

Anne M. Trainor

**ABSTRACT**

Riparian wetlands are complex ecosystems containing great species diversity that may easily be affected by anthropogenic disturbances. Preble's meadow jumping mouse (*Zapus hudsonius preblei*) is a federally threatened species dependent upon riparian wetlands. It has been the subject of habitat management and conservation efforts involving restoration and mitigation projects along the eastern Front Range of Colorado and southeastern Wyoming. Although habitat improvements for *Z. h. preblei* are designed for multiple spatial scales, most knowledge about the species' habitat requirements has been described at a broad landscape scale. In addition, few projects have directly evaluated the mouse's response to restoration and mitigation projects.

The first objective of this study was to determine how supplementation using artificial resources influences the spatial movement patterns of a *Z. h. preblei* population. Previous studies described *Z. h. preblei* use areas through live trapping. This study more precisely evaluated *Z. h. preblei* spatial use by applying radio telemetry within a riparian ecosystem. I conducted an experiment by constructing treatment plots of artificial resources (food and cover) in areas with no previous detections of *Z. h. preblei* during 3 prior years (1998-2000) of intensive monitoring. *Z. h. preblei* were radio collared and then located hourly during nightly activity periods before and after the addition of food and cover. The second objective of this study was to improve understanding about micro-habitat characteristics that *Z. h. preblei* use.

During the resource supplementation experiment, *Z. h. preblei* response to treatment plots varied by year with only 1 of 13 radio-tagged individuals using supplemental resources during 2002 and 6 of 8 in 2003. The lower use in 2002 may have been due to drought conditions, which decreased available herbaceous cover and thus protection from predators. While treatment plot use increased in 2003, the overall use was relatively low when compared to natural, high-use areas. The mean proportion of treatment plot use in 2003 was = 5.9% (SE = 1.4%, range = 0% to 12%). Limited use of treatment plots may have been due to site fidelity and minimal exploratory movements by *Z. h. preblei* or to elevated predation risk.

A comparison of micro-habitat characteristics from random samples of high-use and no-use areas indicated that areas used intensely by *Z. h. preblei* were closer to the center of the creek bed and positively associated with shrub, grass, and woody debris cover. Distance to center of the creek bed, percent shrub cover, and grass cover had the greatest relative importance of the habitat variables modeled in describing high-use areas. High-use areas contained three times the percent of grass cover as forb cover. There was a greater proportion of wetland shrub and grass cover in high-use versus no-use cells. However, proportion of cover type (shrub or grass) did not vary greatly between high-use and no use cells.

Within riparian wetlands, the identification of key micro-habitat components that are intensively used by *Z. h. preblei* could improve conservation and management programs. In addition, results from the resource supplementation experiment suggest that  $TP \hat{p}$  mitigation and restoration may not ensure use of areas by threatened and endangered species. Therefore, understanding how species respond to changes in



areas where they currently live will require development of more efficient and effective mitigation projects, and monitoring by conservation biologists and wildlife managers will be essential.

Prepared by \_\_\_\_\_  
Anne M. Trainor, Colorado State University



### JOB PROGRESS REPORT

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

Period Covered: July 1, 2003 - June 30, 2004

Author: Tanya M. Shenk

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**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

In an effort to establish a viable population of lynx (*Lynx canadensis*) in Colorado, a reintroduction effort was initiated in 1999. The reintroduction effort was augmented with the release of 37 additional animals in April 2004, bringing the total to 166 lynx reintroduced to southwestern Colorado. Each lynx is released with dual satellite and VHF radio transmitters to allow intensive monitoring of animals after release. 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. Locations of each lynx were collected through aerial- or satellite-tracking to document movement patterns. Most lynx remain in the southwestern quarter of Colorado. Reproduction was first documented during the 2003 reproduction season. A second successful breeding season was documented in 2004 with 11 dens and 30 kittens found as of June 30, 2004. Snow-tracking results 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. 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 Colorado conditions 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.

**JOB PROGRESS REPORT**  
**POST-RELEASE MONITORING OF LYNX (*Lynx canadensis*) REINTRODUCED TO**  
**COLORADO**

Tanya M. Shenk

**SEGMENT OBJECTIVES**

The initial post-release monitoring of reintroduced lynx 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.

**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. Given the isolation of Colorado to the nearest northern populations, the Colorado Division of Wildlife (CDOW) considered reintroduction as the only option to attempt to reestablish the species in the state.

A reintroduction effort was begun in 1999. To date, 166 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 seven 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 habitats used. The second primary goal of the monitoring program is to

estimate survival of the reintroduced lynx and, where possible, determine cause of mortality of reintroduced lynx. Such information will help in assessing and modifying release protocols and management of lynx once they have been released.

Additional goals of the post-release monitoring program for lynx reintroduced to the southern Rocky Mountains include refining descriptions of habitat use and movement patterns, determining hunting habits, and obtaining information on reproduction. When the lynx establish home ranges that encompass their preferred habitat, more emphasis will be placed on refining descriptions of movement patterns and habitat use.

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). As a listed species, an additional objective of the post-release monitoring program is to develop conservation strategies relevant to lynx in Colorado. Therefore, information specific to the ecology of the lynx in its southern Rocky Mountain range such as habitats used, movement patterns, mortality factors, survival, and reproduction in Colorado is needed.

## METHODS

### *Reintroduction Effort*

All 2004 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 selected based on land ownership and accessibility during times of release. Lynx were transported from the holding facility to the 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.

### *Movement Patterns*

All lynx released in spring 2004 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 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 and general habitat descriptions at each location were recorded and mapped.

### *Survival and Mortality Factors*

When a mortality signal (75 ppm vs. 50 ppm 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 immediately to the Colorado State University Veterinary Hospital 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.). 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.

*Reproduction*

Females were monitored for proximity to males during breeding season and for site fidelity to a given area during the denning period of May and June. Each female that exhibited stationary movement patterns in May or June 2004 was observed to look for accompanying kittens.

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.

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.

*Diet*

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; the remainder was left in place in the event that the scat was being used by the animal as a territory mark.

**RESULTS**

*Reintroduction Effort*

Based on the adoption of the approved augmentation management strategy (Shenk 2003), 37 lynx (17 females and 20 males) were released in April 2004, bringing the total number of lynx reintroduced to Colorado to 166 (Table 1). Lynx for the 2004 augmentation were captured in Quebec and British Columbia. All 37 lynx were released at previously used release sites in southwestern Colorado.

Table 1. Colorado lynx reintroduction effort as of June 30, 2004.

Year	Females	Males	TOTAL
1999	22	19	41
2000	35	20	55
2003	17	16	33
2004	17	20	37
TOTAL	91	75	166

*Movement Patterns*

Most of the lynx released in 2004 have remained in the southwestern quarter of Colorado, with the exception of 2 lynx that went briefly to New Mexico but subsequently returned to 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. There are some lynx north of Gunnison up to the I70 corridor and in the Taylor Park area. No lynx are known to be north of I70 at this time.

*Mortalities*

Of the total 166 adult lynx released in 1999, 2000, 2003 and 2004 we have 56 known mortalities. Of these 56 mortalities, 25 are from the 1999 releases, 23 are from the 2000 releases, 4 are from the 2003 releases, and 4 are from the 2004 releases. Causes of death are listed in Table 2. Of the 16 kittens known to have been born in Colorado in 2003, we have 7 confirmed mortalities and 3 possible mortalities.

Table 2. Causes of death for adult lynx released into southwestern Colorado in 1999, 2000, 2003 and 2004.

Cause of death	1999		2000		U	2003		2004		Total
	M	F	M	F		M	F	M	F	
Starvation	1	6	1	1						9
Hit by Vehicle		2		3	1				1	7
Shot	3	1	1	1		1				7
Probable Predation		1								1
Plague				4						4
Unknown: Human Caused				1						1
Probable Shot	1	2	1							4
Probable Hit by Vehicle		2								2
Unknown: Not Starvation	1	2		1				1	1	6
Unknown	2	1	4	4		2	1			14
Human Caused								1		1
Total	8	17	7	15	1	3	1	2	2	56

As of June 30, 2004, CDOW was actively tracking 85 of the 110 lynx still possibly alive (Table 3). Of the remaining 25 remaining lynx possibly alive, 24 were ‘missing’ as of June 30, 2004 (Table 3). A lynx was listed as missing if a signal has not been heard from the animal for at least 1 year. One of these missing lynx is the unknown mortality, thus only 23 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 lynx released in 2000 are thought to have slipped their collars. Thus, CDOW tracked 85 individual lynx since at least June 30, 2003.

Table 3. Status of adult lynx reintroduced to Colorado as of June 30, 2004.

	Females	Males	Unknown	TOTALS
Released	91	75		166
Known Dead	35	20	1	56
Possible Alive	56	55		110
Missing	9	15		23 (1 is unknown mortality)
Slipped Collar	1	1?		1-2
Tracking	46	39		85

*Reproduction*

Of the 6 females that had kittens in 2003, 1 died and 2 had collars that shut off prior to denning season in 2004. Of the 3 that could be monitored during the 2004 denning season, 1 had a litter of 2 kittens (YK00F10), 1 did not have kittens (BC00F08) and it is highly probable the third female (YK99F1) has kittens with her based on her movement patterns. We are still trying to document her status.

The 2004 dens were scattered throughout Colorado and 1 den was found in southern Wyoming. Most of the dens were in Engelmann spruce/subalpine fir forests in areas of extensive downfall. Elevations at the den sites ranged from 2652-3560 m (8701 - 11,680 feet). We weighed, photographed, and PIT-tagged the kittens and recorded sex. We also took hair samples from the kittens for genetic work in an attempt to confirm paternity. We processed the kittens as quickly as possible (15-35 minutes) to minimize the time the kittens were without their mother. 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.

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. Snow-trackers documented at least 1 snowshoe hare kill by a kitten in winter 2003-04. The mortality of the female YK00F16 and her 1 kitten 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 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 (Table 4). Although we tried, we were not able to capture any of the 6 surviving kittens to fit them with radio collars. Unless we capture or find any of these kittens from other methods we will not know their fate beyond this 10 months of survival.



Table 4. Known reproduction for summer 2003 and subsequent kitten fates by April 2004.

Female	Release Year	Date Den Found	Kittens Born			Kittens Known Alive in April 2004	Kittens Known Dead in April 2004
			Females	Males	Total		
BC00F8	2000	5/21/2003	?	?	2	1	1
BC00F19	2000	5/26/2003	1	1	2	1	1
YK00F16	2000	6/19/2003	1	1	2	0	2
YK99F1	1999	6/10/2003	2	1	3	2	1
YK00F19	2000	6/11/2003	1	2	3	?	3?
YK00F10	2000	5/31/2003	2	2	4	2	2
TOTAL					16	6	7, 3?

In spring 2004 we had 26 females from the releases in 1999, 2000 and 2003 that had active radio collars. We documented 18 possible mating pairs of lynx during 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. All 4 of the females that had kittens with them through winter 2003-04 bred again this spring, 2 with the same male they successfully bred with last spring.

During May-June 2004 we found 11 dens and a total of 30 kittens (Table 5). Dens were found when we walked in on females that exhibited virtually no movement for at least 10 days from both aerial and ground telemetry. At all dens the females appeared in excellent condition, as did the kittens. The kittens weighed from 250-770 grams. Lynx kittens weigh approximately 200 grams at birth and do not open their eyes until they are 10-17 days old. Three of the 11 females with kittens were from the 2003 releases (Table 5).

Table 5. Lynx reproduction documented in 2004.

Female	Release Year	Date Den Found	Kittens Born		
			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	1	1	2
BC03F09	2003	6/29/2004	1	1	2
YK00F7	2000	6/30/2004	1	1	2
TOTAL			18	12	30

### Diet

Winter diet of lynx was documented through detection of kills found through snow-tracking. In each winter, the most common prey item was snowshoe hare (*Lepus americanus*), followed by red squirrel (*Tamiasciurus hudsonicus*) (Table 6).

Table 6. 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	<i>n</i>	Prey (%)			
		Snowshoe Hare	Red Squirrel	Cottontail	Other
1999	9	55.56	22.22	0	22.22
1999-2000	81	67.46	19.27	1.20	12.05
2000-2001	88	67.41	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	67.56	27.03	2.70	2.70

## DISCUSSION

In an effort to establish a viable population of lynx in Colorado, a reintroduction effort was initiated in 1999. The reintroduction effort was augmented with the release of 37 additional animals in April 2004, bringing the total to 166 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. Human-caused mortality factors such as gunshot and vehicle collision are currently the highest causes of death.

Reproduction was first documented from the 2003 reproduction season. A second successful breeding season was documented in 2004 with 11 dens and 30 kittens found as of June 30, 2004. Live births are the first step towards recruitment. Recruitment into a population would require these kittens to survive through their first year of life and 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 add more so that the population can grow.

Snow-tracking of released lynx provided preliminary information on hunting behavior by documenting location of kills, food caches, chases, and diet composition estimated through scat analysis. 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. Site-scale habitat data collected from snow-tracking efforts indicate Engelmann spruce and subalpine fir are the most common forest stands used by lynx in southwestern Colorado.

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 Colorado conditions 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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Prepared by \_\_\_\_\_  
Tanya M. Shenk, Wildlife Researcher



Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. \_\_\_\_\_ : Mammals Research  
Work Package No. 0670 : Lynx Conservation  
Task No. 2 : Ecology of Snowshoe Hares (*Lepus*  
*americanus*) in Colorado  
Federal Aid Project: N/A :

Period Covered: July 1, 2003 – June 30, 2004

Author: Jennifer L. Zahratka

Personnel: S. W. Buskirk , T. M. Shenk

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### ABSTRACT

A thesis, entitled ‘The population and habitat ecology of snowshoe hares (*Lepus americanus*) in the southern Rocky Mountains’ was completed and submitted to the University of Wyoming in partial fulfillment of a Master of Science degree. The thesis is available from The Colorado Division of Wildlife Library or the University of Wyoming Library. Included in this report is an abstract of the thesis.

## JOB PROGRESS REPORT

### THE POPULATION AND HABITAT ECOLOGY OF SNOWSHOE HARES (*Lepus americanus*) IN THE SOUTHERN ROCKY MOUNTAINS

Jennifer L. Zahratka

#### ABSTRACT

To better understand the population ecology and habitat associations of snowshoe hares (*Lepus americanus*), I studied snowshoe hares in southwestern Colorado in winters 2002 and 2003. I estimated densities from mark-recapture data and compared vegetative attributes in the mature structural stage (SS 4) among three stand types: Engelmann spruce (*Picea engelmannii*)–subalpine fir (*Abies lasiocarpa*), lodgepole pine (*Pinus contorta*), and ponderosa pine (*Pinus ponderosa*).

I used three methods to calculate a boundary strip width ( $W$ ) to estimate the effective area trapped  $\hat{A}(W)$  in order to illustrate the effect of different methods of estimating  $\hat{A}(W)$  on estimates of density. Density estimates [ $\hat{D} = \hat{N} / \hat{A}(W)$ ] in mature spruce-fir ranged from  $0.1 \pm 0.03$  (SE) hares/ha to  $0.9 \pm 0.1$  hares/ha in 2002 and  $0.3 \pm 0.05$  to  $1.0 \pm 0.1$  hares/ha in 2003. I report only minimum number alive (MNA) in lodgepole pine due to too few captures to estimate density. No snowshoe hares were captured in mature ponderosa pine stands.

Model selection based upon the corrected Akaike's Information Criterion (AICc) showed a strong relationship between MNA and understory cover, density of woody stems 1-7 cm in diameter, and the availability of suitable woody stems for food among the mature stand types I studied ( $R = 0.91$ ,  $df = 8$ ,  $P = 0.008$ ). My empirical data support the assumption that snowshoe hares select habitat with protection from predation. However, the availability of suitable woody stems for food is also an important vegetative attribute for hare habitat. Snowshoe hares selected for spruce-fir among the mature stand types I studied. Mature spruce-fir provided more understory, greater density of woody stems 1-7 cm in diameter, and more woody stems (<1.5 cm) for food. In my study, the winter diet of snowshoe hares was overwhelmingly gymnosperms. Extremely low temperatures affected capture success, but moon phase did not.

Counts of fecal pellets are an attractive tool to estimate densities of snowshoe hares because they are less costly and less labor-intensive than conventional mark-recapture techniques. In the southern Rocky Mountains, snowshoe hares and mountain cottontails (*Sylvilagus nuttallii*) are syntopic. Indeed, I captured two mountain cottontails in two traps in which I captured snowshoe hares. Therefore, distinguishing between fecal pellets is necessary for making inferences specific to these species.

Methods to distinguish between the two leporid species have been developed based upon the assumption that the larger snowshoe hare produces larger fecal pellets than the smaller mountain cottontail. In this study, I measured 655 fecal pellets from 10 individual mountain cottontails and 2,374 fecal pellets from 23 individual snowshoe hares: I found no apparent relationship between the body weight of mountain cottontails or snowshoe hares and the size of their fecal pellets (mountain cottontails:  $r = 0.04$ ,  $F = 0.01$ ,  $P = 0.91$ ; snowshoe hares:  $r = 0.48$ ,  $F = 9.3$ ,  $P = 0.005$ ). Although the two species differed in the size of their fecal pellets, the difference (1.2 mm) would be indistinguishable without measuring equipment and is only applicable to adults. While fecal pellet counts may be accurately used to estimate densities of snowshoe hares in the boreal forests of Canada, in the southern Rocky Mountains where leporid species are potentially syntopic, this method may yield misleading results.

Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. \_\_\_\_\_ : Mammals Research  
Work Package No. 0880 : Black-Footed Ferret Conservation  
Task \_\_\_\_\_ : Black-Footed Ferret Recovery Program Disease  
Monitoring & Management

Federal Aid Project N/A :

Period Covered: July 1 2003 through June 30, 2004

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### ABSTRACT

We continued monitoring carnivores at proposed black-footed ferret reintroduction sites for serological evidence of select disease epidemics. Sampling at the Wolf Creek Management Area (WCMA) in August 2004 revealed little evidence of ongoing epidemics that could impede black-footed ferret restoration efforts. Serology data from culled coyotes showed no evidence of active canine distemper or plague epidemics in the WCMA vicinity. In contrast, serologic evidence of exposure to tularemia continues to be relatively high (~30%), consistent with previous observations in this and other monitored areas. We will continue this work as part of the ongoing Colorado–Utah black-footed ferret reintroduction protocol.

**JOB PROGRESS REPORT  
BLACK-FOOTED FERRET RECOVERY PROGRAM DISEASE MONITORING &  
MANAGEMENT**

LISA L. WOLFE

**INTRODUCTION**

As part of the Colorado–Utah black-footed ferret reintroduction protocol, we continued monitoring carnivores at proposed ferret reintroduction sites for serological evidence of select disease epidemics. Originally, we monitored coyote (*Canis latrans*) populations at two Colorado sites: the Little Snake Management Area (LSMA) and the Wolf Creek Management Area (WCMA), Colorado. Under this program, >200 coyotes have been collected for post-mortem examination and samples collected as described in established protocols since March 1997. Monitoring has been accomplished via cooperative efforts of Colorado Division of Wildlife, USDA Wildlife Services, and Bureau of Land Management (BLM) personnel.

To date, no lesions indicative of active infections with any of the select pathogens (*Francisella tularensis*, *Yersinia pestis*, canine distemper virus [CDV]) have been noted on gross examinations of carcasses. However, relatively high proportions (31-89%) of the coyotes collected from the LSMA had positive titers to plague between March 1997 and July 1999. Although the proportion of plague-positive coyotes declined during the sampling period, evidence of continued exposure and perhaps declining prairie dog abundance led to abandonment of surveillance at LSMA after 1999. Monitoring at the WCMA has continued, and black-footed ferrets were reintroduced at this site in 2001.

**RESULTS AND DISCUSSION**

**Disease surveillance**

As part of the Colorado-Utah black-footed ferret reintroduction protocol, we monitored serological evidence of exposure to select infectious diseases in coyotes at Wolf Creek Management Area (WCMA), Colorado; our strategy was to use exposed coyotes as sentinels for detecting epidemics at restoration and prospective release sites. Over 350 coyotes (*Canis latrans*) have been collected for post-mortem examination and samples collected as described in established protocols since March 1997 via cooperative efforts of Colorado Division of Wildlife, USDA Wildlife Services, and Bureau of Land Management (BLM) personnel. Coyotes were collected using a combination of calling and aerial gunning. In 2004, 20 coyotes were sampled (5 pups, 6 juvenile, 6 adult, 3 not aged) in late July. (Because data from juveniles are most useful in detecting evidence of recent epidemics, we discontinued mid-winter sampling in 2002.)

No lesions indicative of active infections with select pathogens (*Francisella tularensis*, *Yersinia pestis*, canine distemper virus [CDV]) were noted on gross examinations of carcasses through 2002; in the absence of meaningful necropsy findings, we discontinued gross examinations of carcasses in 2003.

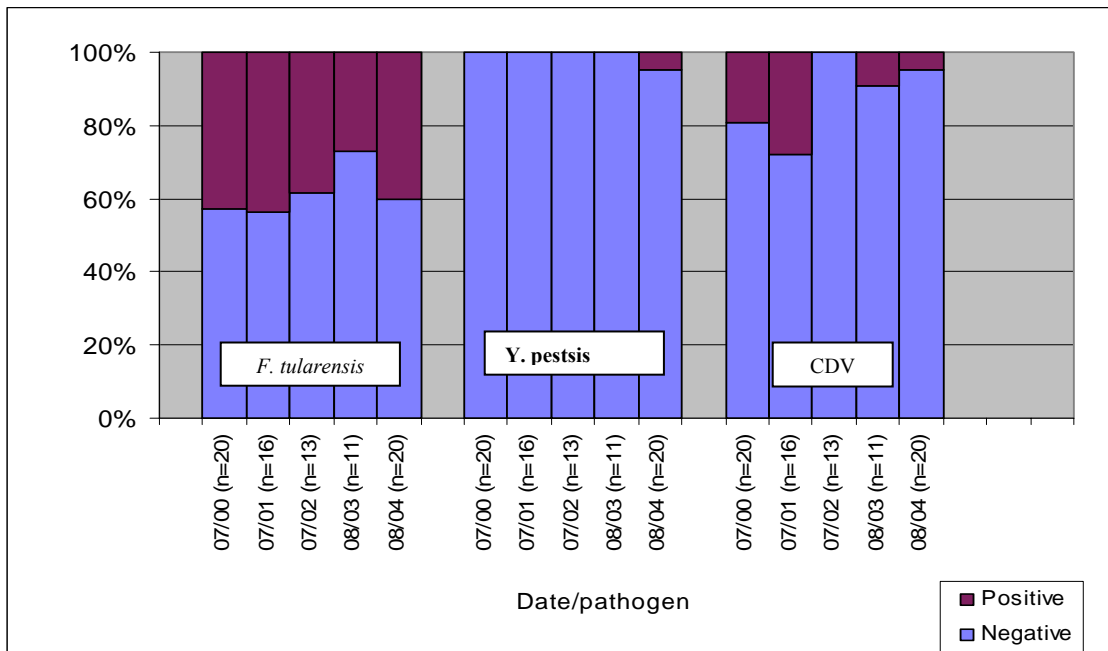
Initial sampling (February 2000) at WCMA indicated substantially lower exposure rates to select pathogens than observed at another site (Little Snake Management Area) monitored in earlier years of this survey. Initial sampling demonstrated 24 percent of the coyotes surveyed with antibody titers suggestive of exposure to CDV. Although seroprevalence increased slightly in 2001, sampling since 2002 revealed much lower proportions of CDV-positive coyotes (Figure 1). There was no serologic evidence of CDV exposure in 2002, and only 1 case each in 2003 and 2004. Exposure to plague still appears relatively rare among coyotes sampled from WCMA (Figure 1). In 2004 one juvenile, out of 21 coyotes sampled, was



“moderately positive” antibody titer to plague. The most significant pathogen exposure noted by seroprevalence is for tularemia. As tularemia is commonly found in rodents in Colorado, seroprevalence of 20–40% is not surprising in carnivores, and very little change in tularemia seroprevalence has been seen over the 5-year sampling period.

Prepared by

Lisa L. Wolfe, Veterinarian



**Figure 1.** Seroprevalence of presumed tularemia (*F. tularensis*), plague (*Y. pestis*), and canine distemper virus (CDV) exposure among coyotes sampled from the Wolf Creek Management Area, Colorado, during summer sampling 2000–2004.



Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

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

Period Covered: July 1, 2003 - June 30, 2004

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### ABSTRACT

To further understand the factors that caused deer numbers to decline in western Colorado during the 1990s, we designed and initiated a field experiment to measure deer population parameters in response to a nutrition enhancement treatment. 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. This 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. 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. We also measured overwinter fawn survival rates in response to the treatment. During 2002 – 2004, we measured pregnancy rates, fetus rates, and body condition of treatment and control adult does during late winter using ultrasonography. We also directly measured fetus survival and neonate survival by using VITs to help locate and radio-collar newborn fawns born from treatment and control does. Estimated percent body fat of adult does during late February and early March, 2002-04,

was significantly 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, we measured a pregnancy rate of 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 (the fetus rate excluding yearlings was 2.00 fetuses/doe, SE = 0.051,  $n = 56$ ). The fetus survival rate with treatment and control fetuses combined was 0.86 (SE = 0.073) during 2002, 0.97 (SE = 0.024) during 2003, and 0.90 (SE = 0.040) during 2004. Fetus survival was similar among treatment and control deer during 2002 – 2003, but not 2004, where treatment fetus survival was 1.00 (SE = 0.000,  $n = 33$ ) and control fetus survival was 0.76 (SE = 0.085,  $n = 25$ ). Based on multiple early winter age classification surveys, we concluded the winter nutrition enhancement treatment did not cause an increase in neonatal production and survival during 2001. However, fawn production and summer-fall survival was relatively good for the overall population, and not representative of most years during the past decade when the population declined. During June – December, 2002–2003, survival of newborn treatment fawns was 0.620 (SE = 0.067) and control fawn survival was 0.493 (SE = 0.070). Survival data coupled with early winter age classification surveys provided evidence the nutrition enhancement treatment increased December fawn recruitment during 2002 and 2003. During December – June, 2001–2004, the overwinter survival rate of fawns was significantly 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 ( $S(t) = 0.655, SE = 0.044$ ). Because of a cross-over experimental design, the treatment unit during winter 2001-02 became the control unit during winters 2002-04, and vice versa. Thus, the treatment effect was replicated across each experimental unit. Combining all years of data, the best model of overwinter fawn survival (AICc = 207.65) included the nutrition enhancement treatment ( $\chi^2_1 = 19.04, P < 0.001$ ), early winter fawn mass ( $\chi^2_1 = 23.27, P < 0.001$ ), and year ( $\chi^2_1 = 6.20, P = 0.045$ ). The AIC model selection analysis emphasized the importance of both the treatment effect as well as early winter mass of fawns, because any models without treatment or fawn mass were poor. Early winter mass of control fawns was slightly higher than that of treatment fawns ( $F_{1,231} = 3.00, P = 0.085$ ); thus the effect of the treatment was not confounded with fawn mass. Data collection will not be completed until January 2005. The results reported here are preliminary and should be treated as such.

**JOB PROGRESS REPORT**  
**EFFECT OF NUTRITION AND HABITAT ENHANCEMENTS ON MULE DEER**  
**RECRUITMENT AND SURVIVAL RATES**

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

**P. N. OBJECTIVES**

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

**SEGMENT OBJECTIVES**

1. Capture and radio-collar a target sample of adult female mule deer and 6 month-old fawns during late November through mid-December in a treatment unit and a control unit.
2. Capture a target sample of adult female mule deer in the treatment unit and the control unit to measure pregnancy rates, fetal rates, and body condition during late February to early March, and fit each adult female deer with a radio collar and vaginal implant transmitter.
3. Deliver the nutrition enhancement treatment to all deer occupying the treatment unit from early December through the end of April.
4. Capture and radio-collar a target sample of newborn fawns from treatment and control radio-collared does during June using the vaginal implant transmitters as a technique to determine the timing and location of birth.
5. Measure fetus survival, neonate survival, early winter fawn:doe ratios, overwinter fawn survival, and annual adult female survival based on radio-collared deer from the treatment and control units.

**INTRODUCTION**

Mule deer (*Odocoileus hemionus*) numbers apparently declined during the 1990's throughout much of the West, and have clearly decreased since the peak population levels documented in the 1940's-60's (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 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. 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 any more or less important than others, and a more comprehensive understanding of multi-factor interactions is needed.

We designed a field experiment to measure deer population responses to nutrition enhancement treatments, to further understand the causative factors underlying observed deer population dynamics. We

are conducting the study on the Uncompahgre Plateau in southwest Colorado, where several predator species are present in abundant numbers: coyotes (*Canis latrans*), mountain lions (*Felis concolor*), and bears (*Ursus americanus*). In addition to predation, myriad diseases in combination proximately affect survival of the Uncompahgre deer population (Pojar and Bowden 2004, B.E. Watkins, unpublished data). Predator numbers have not and will not be manipulated in any manner during the course of the study. All factors have been left constant with the exception of deer nutrition. Deer nutrition is being enhanced by providing supplemental feed to deer occupying a treatment area during the winter. If December fawn recruitment and/or overwinter fawn survival improve as a direct result of the nutrition enhancement treatment, then we can presume that deer nutrition is ultimately more limiting than predation or disease. The second phase of the field experiment, which has not yet been initiated, 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 will evaluate whether nutritional quality of winter range habitat is ultimately more limiting than other factors in a late-seral pinyon-juniper/sagebrush (*Artemisia* spp.) landscape, and if so, whether habitat can be effectively improved for mule deer. The results will also advance our current understanding of multi-factor interactions, with direct implications for mule deer management.

## MATERIALS AND METHODS

### Experimental Approach

#### *Experimental Design and Study Area*

We non-randomly selected two areas within mule deer winter range on the Uncompahgre Plateau to create 2 experimental units (A-B) (Fig. 1). The following criteria were used to select experimental units:

- 1.) Deer densities (~50-80 deer/mi<sup>2</sup>): areas were selected where deer densities were sufficient to meet sample size requirements within the experimental unit, while simultaneously selecting areas that would require feeding less than ~500-600 animals during a normal winter
- 2.) Buffer zones: areas were selected 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: areas were selected that comprise relatively similar habitat complexes and deer densities that are representative of the overall area
- 4.) Elk populations: areas were selected to minimize the number of elk present during normal winters

Units A and B are receiving the nutrition enhancement treatment in a cross-over experimental design, and are being used 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). Beginning November 2002, Unit B received the treatment while Unit A served as the control. Upon completion of P.N. Objective 1, two additional winter range experimental units will be used to conduct phase 2 of the research, or P.N. Objective 2. Habitat in one unit will be manipulated to set back plant succession (treatment), while habitat in the other unit will remain unchanged (control) throughout the experiment.

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 are located in winter range habitat on the Uncompahgre Plateau in southwest Colorado. The nutrition enhancement cross-over design will encompass 4 years.

The 2 experimental units (A and B) receiving the nutrition enhancement treatment are (Figs. 2 and 3):

- (1) Experimental unit A includes 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 is located within the Colona USGS 7.5 Minute Quadrangle, and roughly includes 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 includes 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 is located within the Dry Creek Basin and Montrose West Quadrangles (USGS 7.5 Minute), and roughly includes 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 migrate to summer range. The summer range study area is defined by movements of the radio-collared deer, which encompass >1000 mi<sup>2</sup> covering the southern portion of the Uncompahgre Plateau and adjacent San Juan Mountains to the south and east (Fig. 2). The summer range study area extends north to the Dry Creek river drainage on the Uncompahgre 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 summer on the Uncompahgre Plateau between Dry Creek to the north and Highway 62 to the south.

Winter range elevations range 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 is dominated by pinyon-juniper with interspersed sagebrush adjacent to agricultural fields in the Shavano and Uncompahgre Valleys. Summer range elevations occupied by deer range 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 are dominated by spruce-fir (*Picea* spp.-*Abies* spp.), aspen (*Populus tremuloides*), ponderosa pine (*Pinus ponderosa*), Gambel oak (*Quercus gambelii*), and to a lesser extent, sagebrush and pinyon-juniper at lower elevations.

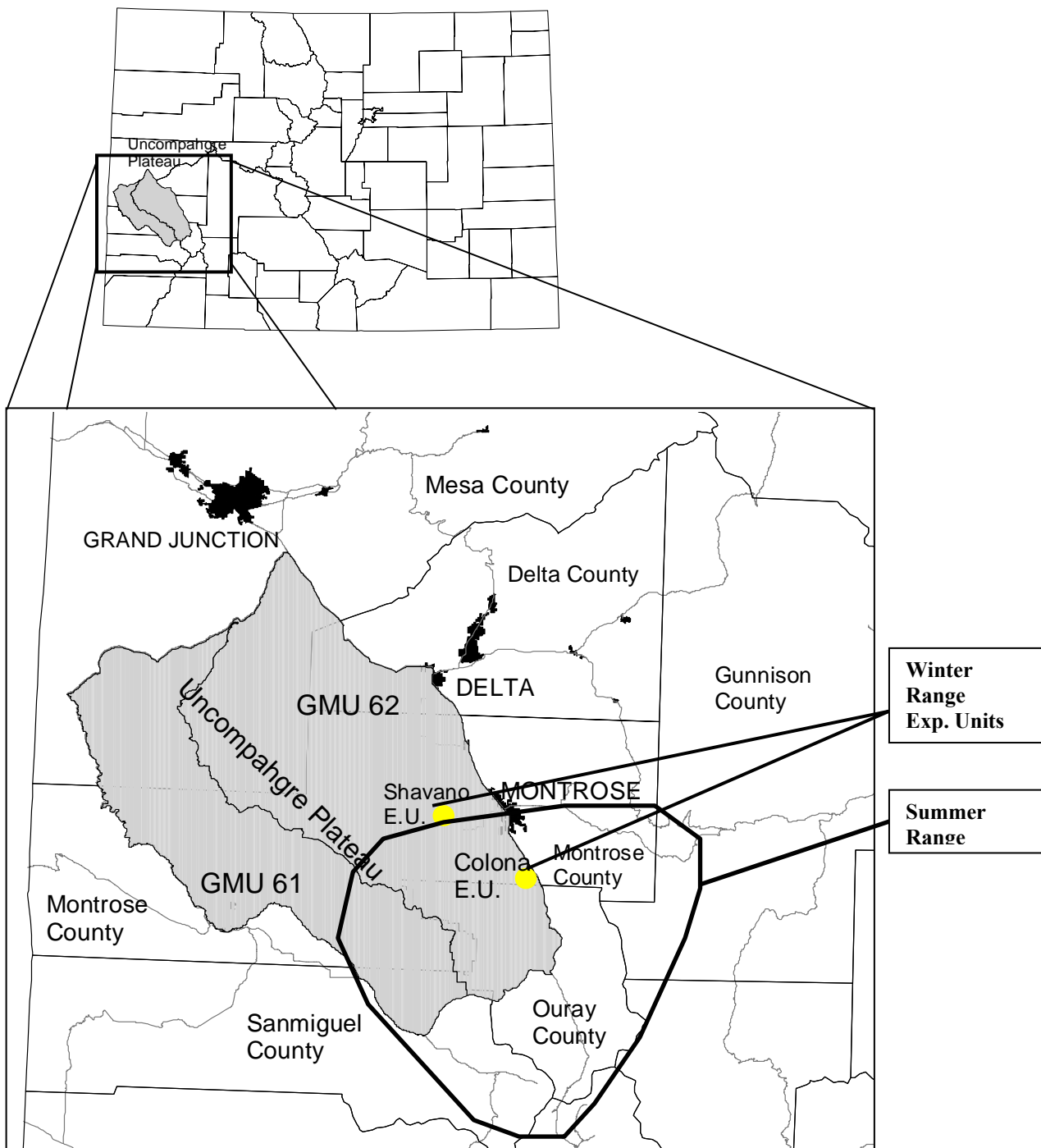


Figure 2. Location of Colona and Shavano (Units A and B) experimental units in Game Management Unit 62 on the Uncompahgre Plateau, southwest Colorado; and location of the summer range study area throughout the southern Uncompahgre Plateau and adjacent San Juan Mountains.



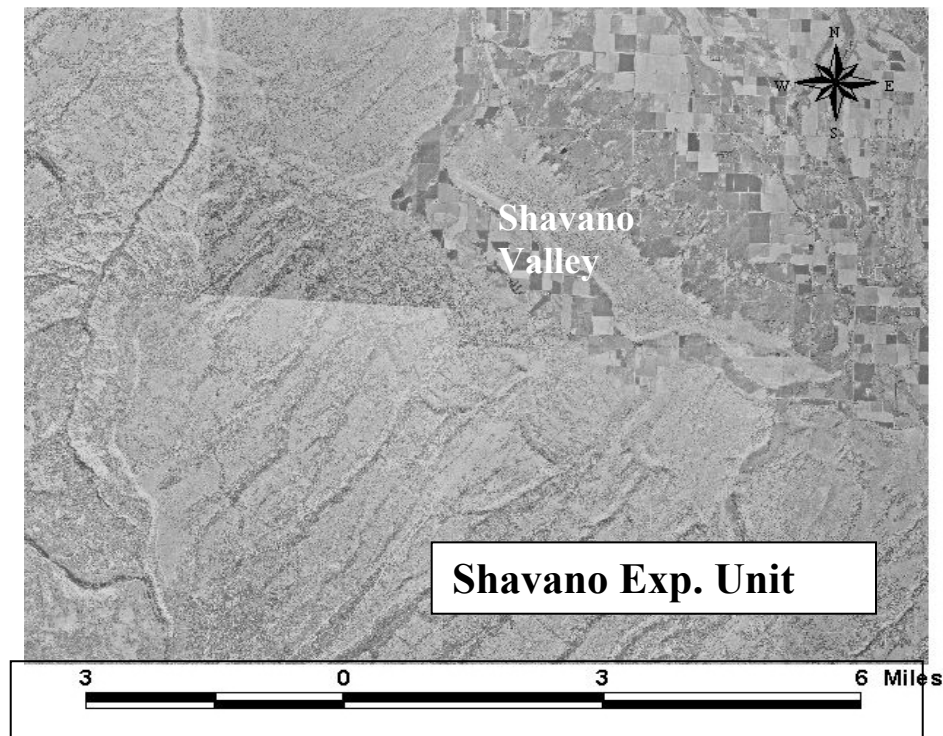
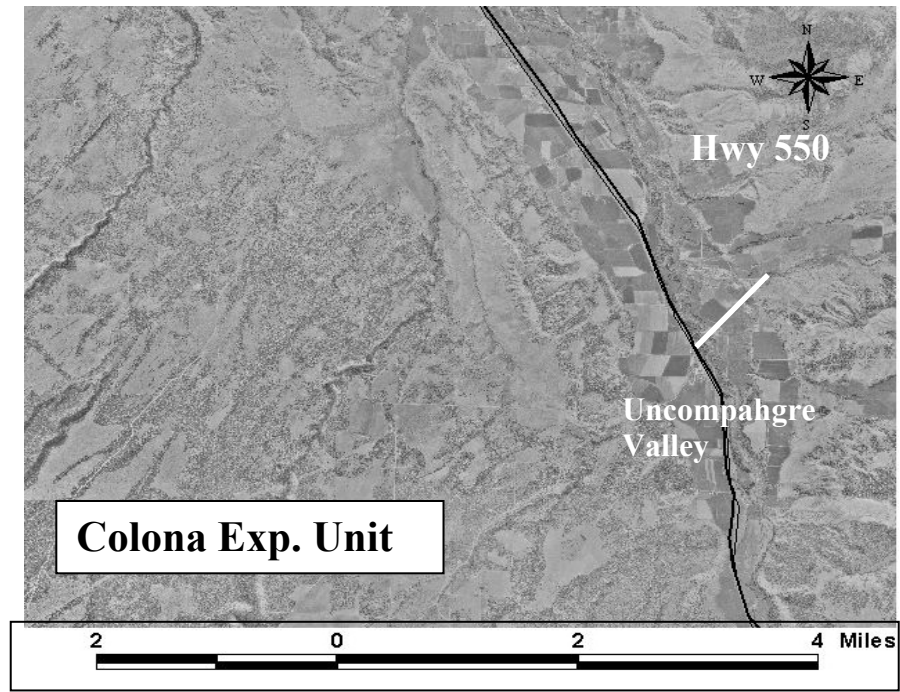


Figure 3. Colona and Shavano experimental units (Units A and B), located in Game Management Unit 62 on the Uncompahgre Plateau, southwest Colorado.

### ***Response Variables***

The response variables are fetal and neonatal survival rates, early winter fawn:doe ratios, and overwinter fawn survival rates. The nutrition enhancement treatment is delivered to deer from December through April, fetus survival is assessed during June, neonate survival is measured from June to December, and fawn:doe ratios are measured during the following December and January (1 year after the treatment was initiated). Overwinter fawn survival is measured from December to June as a direct result of the current winter's treatment. We are measuring these response variables in each experimental unit (treatment and control) to determine whether enhanced winter nutrition of adult does increases subsequent newborn fawn production and survival, and whether enhanced winter nutrition of 6-mo. old fawns directly increases overwinter fawn survival. Ultimately, these measurements provide an assessment of the effect of winter range habitat quality on yearling recruitment, and thus population productivity. We are also measuring overwinter and annual survival of adult does as a function of enhanced winter nutrition.

### ***Sample Size***

Fetus/Neonate Survival: We were primarily interested in survival of newborn fawns from radio-collared does that occupy the 2 winter range experimental units. Fetus survival is also important, but difficult to measure. Fetus rates from a sample of radio-collared does can be measured in winter, but the fate of *all* fetuses cannot be determined the following June because of logistical constraints. Fetus survival rates can only be measured from some unpredictable fraction of the radio-collared doe sample, making sample size calculations of limited use. Thus, our sample size calculations were based on quantifying neonate survival, not fetus survival. For neonate survival, a sample size of 40 neonates per experimental unit per year provides power of 0.81 to detect a difference of 0.15 in survival between 2 experimental units if survival among control fawns is 0.40. We assumed a control survival rate of 0.40 based on neonate survival rates measured recently for the Uncompahgre deer population (Pojar and Bowden 2004) in combination with December fawn:doe ratios measured during the late 1980's and 1990's, when the Uncompahgre population declined (B. E. Watkins, unpublished data). 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 are also being used to evaluate fetus survival; however, logistical constraints limit the power of fetus survival comparisons among experimental units.

Early winter fawn:doe ratios: 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 simple 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 1990's, the standard deviation of the fawn:doe 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 fawn:doe ratio for 40 radio-collared does is  $57/(40^{1/2}) = 9.0$ , which is the expected standard deviation of measured fawn:doe 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 are being 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 is about 0.87.

A sample size of 40 fawns per experimental unit per year provides a power of 0.81 to detect a difference of 0.15 in survival between 2 experimental units if survival on the control unit is 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).

### **Adult and 6-month Old Fawn Capture**

During November and December, adult does and 6-month old fawns were captured using baited drop nets (Ramsey 1968, Schmidt et al. 1978) and helicopter net guns (Barrett et al. 1982, van Reenen 1982). Drop nets were baited with certified weed-free alfalfa hay and apple pulp. Drop nets were used as the principle capture technique for a 3-4 week capture period; helicopter net-gunning was then used 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. Deer captured via drop nets were carried away from the net to an adjacent handling site using stretchers. Deer were fitted with leather radio collars equipped with mortality sensors, which cause an increase in pulse rate after remaining motionless for 4 hours. Permanent collars were placed on adult females, while temporary collars were placed on fawns. To make collars temporary, one end of the collar was cut in half and reattached using rubber surgical tubing; fawns shed the collars  $\geq 6$  months post-capture. A rectangular piece of flexible plastic (Ritchey<sup>®</sup> neck band material) engraved with a unique identifier was stitched 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. The identifiers were necessary to visually identify deer from the ground. This allowed us to effectively document use of the treatment, measure fawn:doe ratios from the ground, and assess experimental unit population size via mark-resight estimators. We recorded the weight, hind foot length and chest girth of each deer, and collected blood samples to evaluate disease prevalence.

During late February and early March, an additional 30 adult female deer were captured 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. For each captured deer, we used ultrasonography to measure pregnancy status, fetal rate, and body condition. Only pregnant does were retained and radio-collared. 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, hind foot length and chest girth 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. Vegetable oil was applied to the shaved area for conduction purposes and fat/muscle thickness was 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):  $\% \text{ Fat} = -6.6387617 + 7.4271417x - 1.11579443x^2 + 0.07733803x^3$  where  $x = \text{rLIVINDEX} = (\text{MAXFAT} - 0.15) + \text{rump BCS}$  (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).

During mid-late winter, we also evaluated differences in serum thyroid hormone concentrations between treatment and control adult does. 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 plastic wings with a width of 57 mm when fully spread apart. The plastic 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/battery 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. The VIT was inserted 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 the appropriate distance it should be inserted into the deer. The length of a typical mule deer vaginal tract was obtained by taking measurements from road-killed deer and/or other fresh deer carcasses obtained in the study area.

Prior to use in the field, VITs were sterilized using a Chlorhexidine solution, air-dried, and sealed in a 3" x 8" sterilization pouch. Sterilization containers with Chlorhexidine solution were used on site during capture to sterilize the vaginoscope and alligator forceps between each use. A new pair of nitrile surgical gloves was used 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 then liberally applied sterile KY Jelly to the scope and inserted it into the deer's vagina to the point where the mark on the applicator was reached. The alligator forceps, which extended through the vaginoscope to hold the VIT, was held firmly in place while the scope was pulled out from the vagina. This procedure pushed the VIT out of the scope into the vagina, and the plastic wings spread apart 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.

### **Neonate Fawn Capture**

During June we relocated each of the radio-collared does having a VIT each morning using aerial and ground telemetry. Flights began at 0530 hr and were usually completed by 1000 – 1100 hrs. The early flights were crucial for detecting fast signals because shed VITs could exceed 90 °F by mid-day if shed in the open, which caused them to switch back to a slow ("pre-birth") pulse. When a fast ("postpartum") pulse rate was detected, we located the VIT from the ground to determine whether it was shed at the birth site. If the transmitter was located at the birth site, we identified whether any fawn(s) were stillborn. If the fawn(s) were no longer present at the birth site, or could not be found in the vicinity of the birth site, we located the radio-collared doe and searched for fawns at her location. All personnel involved wore surgical gloves to help minimize human scent when handling fawns. For each doe, we attempted to locate each of her fawns and document whether any fawns were stillborn. We attempted to account for each doe's fetuses in order to quantify *in utero* fetal survival from February to birth. 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, leather tabs extended from the end of the elastic and from the transmitter for attachment purposes. Collars were made temporary by cutting the leather tab extending from the elastic and reattaching the leather with latex tubing, which caused the collars to shed from the animal >6 months post-capture. For each fawn, mass and hind foot length 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 also routinely located and attempted to capture fawns from treatment and control radio-collared does not having VITs to help achieve our targeted sample size. Each of these does had been previously captured during the research, and were present on either the treatment or control experimental unit during winter.

### **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 (live/mortality). We also attempted to determine the cause of each mortality, with a primary goal of distinguishing between predation and non-predation mortality causes. Deer were radio-monitored from the ground on a daily basis throughout the year and from the air on approximately a biweekly basis. We were able to detect 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 is 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**

Deer nutrition was enhanced in the treatment area 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. Pellets were distributed 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. Each 50lb. bag of pellets was carried  $\leq 200\text{m}$  from the vehicle and distributed by hand in approximately 20-30 small piles of feed in a linear fashion. Numerous bags were

distributed in successive order allowing us to create linear lines of feed that spanned most of the treatment area, which prevented animals from concentrating in any single location. This feeding technique also prevented dominant animals from restricting access to the food supply because of the large area over which pellets were distributed. We supplied pellets ad libitum where a small residual remained when the next day's ration was provided. Collared deer were closely monitored 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 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. This was because some fawns died shortly after capture (e.g. 2-3 weeks), before we could document whether they had access to the feed. 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 constituents (i.e. digestibility, protein, gross energy etc.) 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 is a limiting factor to recruitment. If nutrition is in fact limiting, we will rely on habitat manipulation treatments to evaluate what exactly can be done via management to increase fawn survival and recruitment.

### **Statistical Methods**

A preliminary fawn:doe ratio analysis was completed 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. Survival rates were calculated using a Kaplan-Meier survival analysis (Kaplan and Meier 1958, Pollock et al. 1989), and contrasted among experimental units and sexes using a chi-square analysis. For neonate survival analyses, we used a common entry date because a staggered entry would have biased survival rates low due to early mortalities that occurred before most of the sample was captured. We modeled overwinter fawn survival with a logistic regression model using PROC LOGISTIC in SAS (SAS Institute 1989a); model selection was performed using Akaike's Information Criterion (AIC) (Burnham and Anderson 1998). Survival was modeled as a function of the nutrition enhancement treatment, sex, year, and capture mass. We used a general linear model in PROC GLM in SAS (SAS Institute 1989b) 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 thyroid hormones between treatment and control does. We then used PROC REG (SAS Institute 1989b) to evaluate the relationship between estimated percent body fat and serum thyroid hormone concentrations. We analyzed fetus survival directly with a binomial survival rate for the subset of fetuses with 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). Other results in this report are presented as data summaries incorporating means and standard errors, or in some cases, raw data values. These results are incomplete and preliminary, and should be treated as such.

## 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*

From December 15, 2000, through April 19, 2001, we distributed 88 tons of the pelleted ration. For most of the winter and spring, on average, we distributed 0.85 tons of feed each day throughout 22 feeding sites across the 2.3 mi<sup>2</sup> treatment unit. Deer were fed ad libitum because there was always residual feed remaining the next day during the feeding routine. Each sack was distributed in approximately 20-30 distinct, small piles, resulting in >1000 small piles of feed throughout the treatment unit. This effort allowed deer to effectively access the feed in small groups, and no aggression was ever observed among deer seeking access to the feed. By distributing the feed in this manner, we were able to avoid the negative aspects associated with large-scale feeding operations. Deer adapted to the pelleted supplement right away 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, indicate 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, indicate 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<sup>2</sup> (SE = 9), but increased shortly after and was 213 deer/mi<sup>2</sup> (SE = 27) in March. Deer densities in the control unit changed little from 83 deer/mi<sup>2</sup> (SE = 12) in December to 101 deer/mi<sup>2</sup> (SE = 14) in March.

#### *2001-02*

From December 15, 2001, through April 25, 2002, we distributed 194 tons of the supplement throughout the treatment unit. For most of the winter and spring, we distributed 2.0-2.1 tons of feed each day. The dramatic increase in supplement distribution from the previous year occurred because a large number of elk descended into the Uncompahgre Valley during mid-late fall/early winter. 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 2.3 mi<sup>2</sup> treatment unit throughout most of the winter.

Given myriad logistical and budgetary constraints, 2.1 tons was the maximum amount of feed we could routinely deliver on a daily basis. 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 the pelleted supplement 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). Most days, >100 and sometimes 200-300 deer were observed utilizing the pellets during the course of distributing the supplement. These observations rarely included elk; thus, deer-elk competition was minimized because of temporal differences in feeding, and deer clearly had first access to the feed.

### **2002-03**

Beginning December 2002, we switched the treatment and control units consistent with the cross-over experimental design. From December 15, 2002, through April 30, 2003, we distributed 97 tons of the supplement throughout the new treatment unit, which had served as the control unit the previous 2 years. The supplement was distributed daily throughout 29 sites over a larger area (~7 mi<sup>2</sup>) 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**

From December 10, 2003, through April 30, 2004, we distributed 197 tons of the supplement throughout the treatment unit. The increase in supplement distribution occurred because of an increase in elk 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. By targeting a portion of the daily feed distribution to elk, we restricted elk to the upper extent of the deer winter range for most of the winter. 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 significantly 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  $\times$  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 ( $1.04 \cdot T4 - 0.02 \cdot T3 + 0.77 \cdot FT4 - 0.17 \cdot FT3$ ). 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 ( $\% \text{ Fat} = -3.122 + 0.090 \cdot T4, r^2 = 0.52, P < 0.001$ ). 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).

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.

Year	Exp. Unit	Thyroid Hormone			
		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)

### Fetus Survival and Pregnancy/Fetus Rates

We began measuring fetus survival in 2002 as part of our effort to capture and radio-collar newborn fawns born from radio-collared does. Similar numbers of stillborns were observed between treatment and control does during both 2002 and 2003, so fetus survival estimates for those years are not differentiated by experimental unit. In February-March 2002, 36 of 38 adult does captured were pregnant, thus the pregnancy rate was 0.95 (SE = 0.036). 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. During June 2002, we determined the fate of all fetuses (live or stillborn) from only 14 of the 36 VIT does, largely because of a high VIT battery failure rate. The survival rate of fetuses ( $n = 22$ ) from these 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).

In February-March 2003, 58 of 63 adult does captured were pregnant, resulting in a pregnancy rate of 0.92 (SE = 0.034). Critical personnel and equipment for measuring fetus rates were not continuously available due to capture delays associated with helicopter mechanical problems. Some of the 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 birthsites during June. We were unable to determine a fetus count for 8 treatment deer because only pregnancy was established with ultrasound and no birthsite 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. During June 2003, we determined the fate of all fetuses (live or stillborn) from 33 of the 58 VIT does; the good success was based on VITs commonly being shed at birthsites. The survival rate of fetuses ( $n = 58$ ) from these 33 does was 0.97 (SE = 0.024). In June 2003, incorporating all does ( $n = 71$ ) that we located any fawn from, 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).

In February 2004, the overall 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. We determined the fate of all fetuses (live or stillborn) from 31 of the 60 VIT does. The overall fetus survival rate was 0.90 (SE = 0.040,  $n = 58$ ). Different from 2002 or 2003, each of these 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.

## **Neonatal Survival/Fawn:Doe Ratios**

### **2001**

In December 2000, at the beginning of the study and prior to the first year's treatment delivery, fawn:doe ratios were similar in the 2 experimental units. Pre-treatment fawn:doe ratios were 52.6 fawns:100 does (SE = 5.3) in the treatment unit, and 51.6 fawns:100 does (SE = 5.0) in the control unit. In late December 2001 and early January 2002, following the first year's treatment, we conducted 2 age classification helicopter surveys in the treatment and control units. On 12/23/01, 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 1/8/02, 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 ( $t_{74} = 1.16$ ,  $P = 0.249$ ).

The fawn:doe ratio results are conflicting, and clearly do not provide evidence that there was any treatment effect. In short, we concluded that the nutrition enhancement treatment did not cause an increase in neonatal production and survival during 2001. However, our results, in conjunction with a December estimate of 64 fawns:100 does for the entire Uncompahgre deer population (B.E. Watkins,

unpublished), indicate fawn production and survival was good during 2001. The observed fawn:doe ratios coupled with overwinter fawn survival and annual adult survival rates indicate the deer population was increasing. Considering the past 1-2 decades, this was an atypically good year for the Uncompahgre deer population.

### **2002**

During June – December 2002, following the second year's treatment, we measured neonate survival directly using radio-collared fawns; however, sample sizes were based on a technique assessment of VITs and were relatively small for contrasting treatment and control survival of neonates (Bishop et al. 2002). Treatment fawn survival 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 12/31/02, 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 1/21/03, 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. Oppositely, 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. Also, survival data combined with age ratio data indicate neonate production and survival was reasonably favorable during 2002, and not indicative of the low fawn recruitment observed during the late 1980's and 1990's.

### **2003**

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 54 does fitted with VITs. Treatment fawn survival (June – 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.

### **2002-03**

Survival rate point estimates were very similar during 2002 and 2003. Combined over both years, treatment survival ( $S(t) = 0.620$ , SE = 0.067) was higher ( $P = 0.189$ ) than control survival ( $S(t) = 0.493$ , SE = 0.070). The high censor rate due to shed collars during fall affected the p-value. Neonate survival through July 15, 2002 and 2003, was significantly higher ( $P = 0.006$ ) for treatment fawns ( $S(t) = 0.833$ , SE = 0.041) than control fawns ( $S(t) = 0.634$ , SE = 0.057). We are currently measuring 2004 neonate survival rates, which will be necessary for final interpretations as to the effectiveness of the treatment.

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 is 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 incorrectly classifying 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 during 2003. Deer were not as concentrated during helicopter surveys, and unlike previous years, almost all of the ground classification data for the Colona experimental unit was obtained by radio-tracking does during the day.

Given the inherent difficulties of measuring fawn:doe ratios in the 2 experimental units, and the lack of a clear indication as to the effectiveness of the treatment, we will only cautiously use fawn:doe ratios to make inferences regarding treatment effects. At the completion of the research, we will test whether enhanced winter nutrition of adult does improved newborn fawn survival based on a three-year model of the radio-collared neonate survival data.

### ***Neonate Mortality Causes***

During June – December of 2002 and 2003, 32 of 84 treatment fawns died: 8 – coyote predation, 2 – bear predation, 2 – felid predation, 3 – predation where the predator was undetermined, 9 – disease/starvation/ malnutrition, 1 – abandonment, 2 – trauma/injury, 1 – road-kill, 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, 10.1% from coyote predation, 3.8% from predation where the predator was undetermined, 2.5% each from bear predation, felid predation, injury/trauma, and unknown causes, and 1.3% each from abandonment and road-kill. Simplified, 18.9% of all treatment fawns died from predation, 11.4% died from disease/starvation/malnutrition, and 7.6% died from other or unknown causes. During June – December of 2002 and 2003, 35 of 72 control fawns died: 12 – coyote predation, 4 – felid predation, 2 – bear predation, 1 – predation where the predator was undetermined, 11 – disease/starvation/ malnutrition, 1 – trauma/injury, and 4 – unknown. Converted to mortality rates based on the Kaplan-Meier survival analysis, 17.4% of all control fawns died from coyote predation, 15.9% died from disease/starvation/malnutrition, 5.8% each from felid predation and unknown causes, 2.9% from bear predation, and 1.4% each from trauma/injury and predation where the predator was undetermined. Simplified, 27.5% of all control fawns died from predation, 15.9% from disease/starvation/malnutrition, and 7.2% from other or unknown causes. In summary, mortality rates due to predation and disease/starvation/malnutrition were lower for treatment fawns than control fawns.

### ***Overwinter Fawn Survival and Mortality Causes***

During winter 2001-02 (Dec 10, 2001 – June 15, 2002), the survival rate of fawns was significantly greater ( $\chi^2_1 = 13.216$ ,  $P < 0.001$ ) 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 (Dec 10, 2002 – June 15, 2003), the overwinter survival rate of fawns was significantly greater ( $\chi^2_1 = 5.734$ ,  $P = 0.017$ ) 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 (Dec 10, 2003 – June 15, 2004), the overwinter survival rate of fawns was significantly greater ( $\chi^2_1 = 3.852$ ,  $P = 0.050$ ) 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

higher ( $\chi^2_1 = 18.781$ ,  $P < 0.001$ ) than control fawn survival ( $S(t) = 0.655$ ,  $SE = 0.044$ ) (Fig. 4). 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. Combining all years of data, the best model of overwinter fawn survival (AICc = 207.65) included treatment ( $\chi^2_1 = 19.04$ ,  $P < 0.001$ ), early winter fawn mass ( $\chi^2_1 = 23.27$ ,  $P < 0.001$ ), and year ( $\chi^2_1 = 6.20$ ,  $P = 0.045$ ). The AIC model selection analysis emphasizes the importance of both the treatment effect as well as early winter mass of fawns, because any models without treatment or fawn mass were very poor (Table 2). Survival of fawns receiving the nutrition enhancement treatment was 0.24 higher than survival of control fawns during three mild to average winters, and surviving fawns averaged 3.5 kg heavier than fawns that died. Early winter mass of control fawns was slightly higher than that of treatment fawns ( $F_{1, 231} = 3.00$ ,  $P = 0.085$ ); thus the effect of the treatment was not confounded with fawn mass. Fawn mass was similar between winters as well ( $F_{2, 231} = 1.31$ ,  $P = 0.273$ ). 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). In summary, the nutrition enhancement treatment improved overwinter fawn survival and thus yearling recruitment, and heavier fawns in each experimental unit had higher survival probabilities.

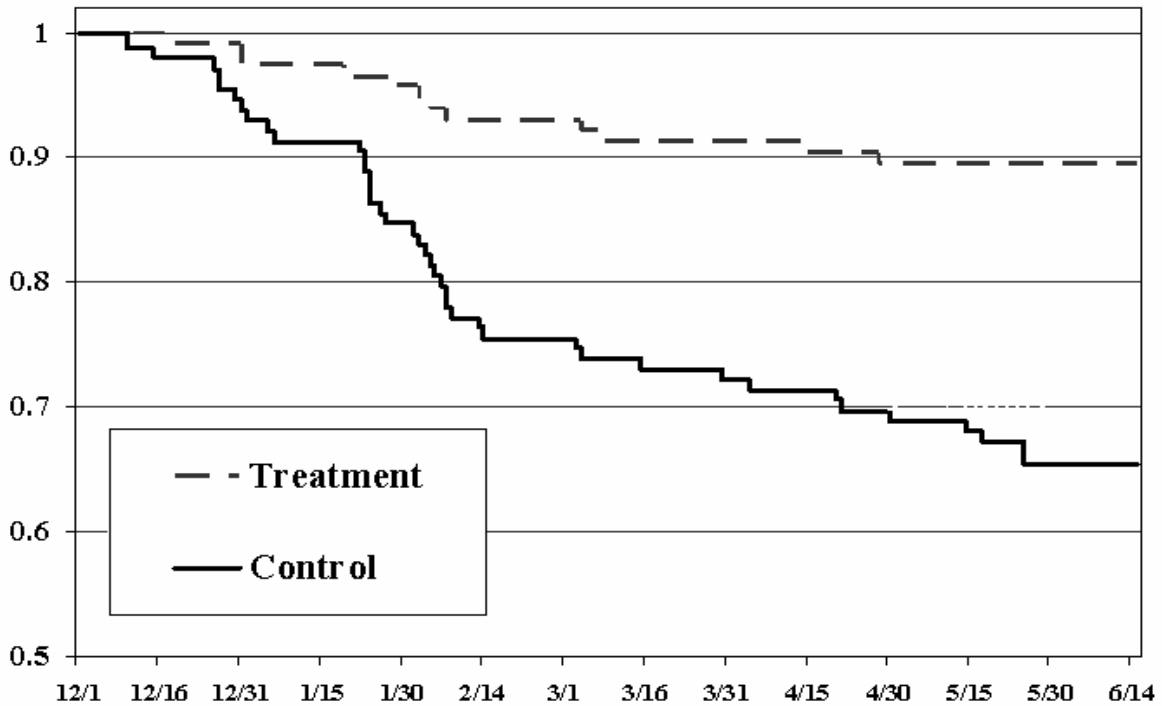


Figure 4. Overwinter fawn survival (Dec 10 – June 15, 2001 – 2004) in a nutrition enhancement treatment unit ( $S(t) = 0.895$ ,  $SE = 0.029$ ) and a control unit ( $S(t) = 0.655$ ,  $SE = 0.044$ ) on the Uncompahgre Plateau, southwest Colorado.

Table 2. Model selection results for a logistic regression analysis of overwinter mule deer fawn survival in southwest Colorado, 2001– 2004. Enhanced nutrition (Treatment) and early winter fawn mass were the critical predictors of survival. Model selection was performed using Akaike’s Information Criterion (AIC).

Model Name	-2 Log Likelihood	# Parameters (K)	AIC	AICc	$\Delta$ AICc
Treatment + Year + Mass	202.254	5	212.254	207.649	0
Treatment + Sex + Year + Mass	201.227	6	213.227	207.783	0.13
Treatment + Sex + Year + Trt*Year + Mass	201.060	8	217.060	210.026	2.38
Treatment + Sex + Mass	207.179	4	215.179	211.440	3.79
Treatment + Mass	208.556	3	214.556	211.712	4.06
Sex + Year + Mass	223.598	5	233.598	228.993	21.34
Treatment	235.739	2	239.739	237.816	30.17
Sex + Year	248.878	4	256.878	253.139	45.49

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. 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. Most fawns killed by predators had little or no femur marrow fat remaining, indicating the predation was likely compensatory in nature.

#### Adult Female Survival and Causes of Mortality

During winter 2000-01 (Dec 1, 2000 – May 31, 2001), the adult doe survival rate in the treatment unit ( $S(t) = 0.968$ ,  $SE = 0.032$ ) was greater ( $\chi^2_1 = 2.649$ ,  $P = 0.104$ ) than the survival rate in the control unit ( $S(t) = 0.861$ ,  $SE = 0.058$ ). However, annual adult doe survival rates (Dec 1, 2000 – Nov 30, 2001) were similar among the treatment and control deer (Trt:  $S(t) = 0.839$ ,  $SE = 0.066$ ; Control:  $S(t) = 0.833$ ,  $SE = 0.062$ ;  $\chi^2_1 = 0.004$ ,  $P = 0.947$ ). 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 ( $\chi^2_1 = 3.116$ ,  $P = 0.078$ ) 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:  $S(t) = 0.818$ ,  $SE = 0.047$ ;  $\chi^2_1 = 0.090$ ,  $P = 0.764$ ). 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:  $S(t) = 0.924$ ,  $SE = 0.028$ ;  $\chi^2_1 = 0.360$ ,  $P = 0.549$ ). 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. However, annual adult doe survival rates (Dec 1, 2002 – Nov 30, 2003) were higher ( $\chi^2_1 = 2.016$ ,  $P = 0.156$ ) for treatment does 0.888 ( $SE = 0.034$ ) than control does 0.813 ( $SE = 0.041$ ). The main difference from the previous 2 years was 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 ( $\chi^2_1 = 3.843$ ,  $P = 0.050$ ) among treatment does ( $S(t) = 0.979$ ,  $SE = 0.014$ ) than control does ( $S(t) = 0.915$ ,  $SE = 0.027$ ). Thus far in 2004, annual adult doe survival rates (Dec 1, 2003 – 8/31, 2004) are 0.951 ( $SE = 0.021$ ) for treatment does and 0.896 ( $SE = 0.029$ ) for control does. Considering all years, the treatment has improved overwinter adult doe survival but had a relatively minor affect on annual survival. Considering only the past 2 years, there is evidence the treatment has had a positive affect on annual survival. Annual survival rates measured in this study align with expected survival based on other studies (Unsworth et al. 1999, B.E. Watkins, unpublished).

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 – injury (fence), 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 Dec 2002 – August 2004, with Shavano as the treatment and Colona as the control, there have been 14 treatment doe mortalities: 5 – disease/infection, 5 unknown causes, 3 – coyote predation, and 1 – road-killed. 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. There have been 26 control adult doe mortalities during this same time period: 7 – malnutrition/disease, 5 – road-kill, 4 – coyote predation, 4 – unknown causes, 3 – mountain lion predation, and 3 – non-predation unknown. Malnutrition, predation, and road-kill were the major mortality factors of control does during 2002-04.

## SUMMARY

We successfully enhanced nutrition of deer occupying the treatment units. There was no evidence the treatment positively influenced fetus survival until 2004, when virtually all stillborn fetuses were from control adult does. We currently have evidence the treatment caused an increase in neonate survival; however, data collection is incomplete. The treatment caused a significant increase in overwinter fawn survival, which is where the greatest differences occurred between treatment and control deer. Overwinter adult doe survival increased as a result of the treatment, but annual survival was more similar among treatment and control adult does. Results reported here are based on preliminary analyses, and in some cases, incomplete data sets. Final analyses will be conducted once data collection is complete.

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Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of	<u>Colorado</u>	:	<u>Cost Center 3430</u>
Project No.	<u>W-153-R</u>	:	<u>Mammals Research</u>
Work Package No.	<u>3002</u>	:	<u>Elk Conservation</u>
Task No.	<u>RMNP</u>	:	<u>Technical Support for Elk and Vegetation Management Environmental Impact Statement for Rocky Mountain National Park</u>
Federal Aid Project:	_____	:	_____

Period Covered : July 1, 2003 - June 30, 2004

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### ABSTRACT

Practical application of fertility control technology in free-ranging wild ungulates requires remote delivery of a safe and efficacious contraceptive agent. The objective of this investigation was to evaluate the potential of a remotely delivered, sustained release, biodegradable implant formulation of leuprolide acetate, to achieve reversible suppression of ovulation and 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 the implant formulation of leuprolide, and the remaining nine elk received the same formulation without leuprolide. We measured pregnancy rates, suppression of luteinizing hormone (LH) and progesterone concentrations, and reversibility of leuprolide treatments during 1 August 2002 to 3 September 2003. The sustained release implant formulation, remotely administered by dart, resulted in decreased concentrations of LH and progesterone, temporary suppression of ovulation and steroidogenesis, and effective contraception (100%) for one breeding season. These results extend the potential for practical application of the leuprolide implant as contraceptive agent in female elk, where in the absence of such technology, wild elk must first be captured and restrained prior to treatment.

**JOB PROGRESS REPORT**  
**TECHNICAL SUPPORT FOR ELK AND VEGETATION MANAGEMENT ENVIRONMENTAL**  
**IMPACT STATEMENT FOR ROCKY MOUNTAIN NATIONAL PARK**

D. L. Baker, M. A. Wild, M. D. Hussain, R. L. Dunn, and T. M. Nett

**P. N. OBJECTIVE**

Conduct experiments with captive and free-ranging elk to evaluate fertility control as a management alternative for controlling elk populations in Rocky Mountain National Park (RMNP), Colorado.

**SEGMENT OBJECTIVES**

1. Determine the effectiveness of a remotely delivered intramuscular leuprolide implant in preventing pregnancy in captive female elk.
2. Determine the duration of effectiveness of remotely delivered leuprolide implant (if any) on luteinizing hormone (LH) and progesterone secretion in captive female elk.
3. Determine the reversibility of remotely delivered leuprolide implant on infertility (if achieved) in captive female elk.

**INTRODUCTION**

Fundamental to practical application of contraceptives to wildlife, is a safe and effective antifertility agent that can be remotely delivered to the target species. To attain this goal, considerable research has focused on the development and testing of ballistic systems and controlled drug release formulations that can remotely administer contraceptive agents to wild ungulates (Kreeger, 1997). Contraceptive agents have been delivered via projectile dart or biodegradable implant to a variety of wild ungulate species including deer (*Odocoileus spp.*) (Turner et al., 1992; Jacobsen et al., 1995; DeNicola et al., 1997), elk (*Cervus elaphus nannodes*) (Shideler et al., 2002), wild horses (*Equus caballus*) (Kirkpatrick et al., 1990), burros (*Equus asinus*) (Turner et al., 1996), and elephants (*Loxodonta africana*) (Delsink et al., 2002). However, to date, no contraceptive agent that possess all of the desired attributes (Fagerstone et al., 2002) has been developed for remote delivery.

The use of GnRH agonist implants to suppress short-term ovarian follicular growth and ovulation are well documented for a number of species including cattle (McLeod et al., 1991, D'Occhio et al., 1996), sheep (McNeilly and Fraser, 1987), monkeys (Fraser et al., 1987), and humans (Broekmans et al., 1996). However, few studies have established the efficacy of these agents for long-term suppression of ovarian activity and contraception (Trigg et al., 2001; Baker et al., 2002, 2004; D'Occhio et al., 2002) and to our knowledge, none have previously demonstrated effective contraception by dart delivery of the implant.

In previous research, we administered gonadotropin releasing hormone (GnRH) agonist leuprolide acetate by hand injection to captive female elk (*Cervus elaphus nelsoni*) (Baker et al., 2002), and mule deer (*Odocoileus hemionus hemionus*) (Baker et al. 2004), as a sustained release injectable implant, and achieved 100 % infertility for one breeding season. The implant formulation consisted of 45 % w/w 75/25 poly (DL-lactide-co-glycolide) (PLG) polymer having an intrinsic viscosity of 0.20 dL/g dissolved in N-methyl-2-pyrrolidone (NMP) and containing 6 % w/w leuprolide in the polymer solution. This formulation was designed to release the drug for a period of 3 to 4 months after subcutaneous injection (Ravivarapu et al., 2000).

In these previous studies, the leuprolide formulation was demonstrated to be highly effective when delivered subcutaneously, however, it's not known if similar effectiveness can be achieved when administered as an intramuscular (IM) injection via dart. Differences in drug pharmacokinetics and metabolism between muscle and subcutaneous tissues could affect release dynamics of the implant and possibly decrease the antifertility properties of leuprolide. Therefore, the objectives of this experiment were to determine in captive female elk (1) the effectiveness of this remotely delivered intramuscular leuprolide implant in preventing pregnancy, (2) the duration of effects (if any) on luteinizing hormone (LH) and progesterone secretion, and (3) the reversibility of infertility (if achieved).

## MATERIALS AND METHODS

### Experimental animals

During 1 August 2002 to 3 September 2003, we evaluated the effects of remotely delivered leuprolide formulation on pregnancy rates, luteinizing hormone (LH), and progesterone secretion in captive female elk. Controlled experiments were conducted with 15 adult females (2-14 years of age; 220 - 275 kg BW), two intact adult male elk (3 years of age; 350-400 kg BW), and one epididymectomized adult male elk (3 years of age; 340-375 kg BW) at the Colorado Division of Wildlife's Foothills Wildlife Research Facility in Fort Collins, Colorado, USA. Captive elk used in this experiment were permanently maintained at this facility and were trained to repeated handling, weighing, blood sampling techniques, and isolation pens. When not involved in the periodic intensive sampling procedures required by this study, elk were maintained in fenced pastures (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.

In an effort to induce normal cyclic ovulatory responses and synchronize estrus, we released an epididymectomized male elk with 15 seasonally anovulatory female elk on 20 July 2002 (McComb, 1987). Four weeks later (21 August) and prior to assigning elk to experimental treatments, we assessed the reproductive status of each female by: 1) manual rectal palpation of the reproductive tract to diagnose ovarian status and identify any abnormalities, and 2) measuring the responsiveness of pituitary gonadotropes to an exogenous dose of GnRH analog. Females showing evidence of reproductive tract abnormalities or suppressed gonadotrope function were excluded from the experiment.

### Experimental design

Fifteen female elk were randomly assigned to one of three experimental groups. Six elk (group A) were injected with a dart containing the polymeric matrix formulation of leuprolide acetate (D-Leu<sup>6</sup>-GnRH-Pro<sup>9</sup>-ethylamide). Four elk (group B) were designated as pregnant controls. They received the polymer solution without leuprolide and were used to compare the effects of leuprolide formulation on pregnancy rates between treated and untreated elk. These two groups of elk were maintained together in the same pastures with two intact, adult male elk from 13 September 2002 to 10 April 2003. The remaining five elk (group C) served as non-pregnant controls and were placed in a separate pasture (2 ha) without direct contact with male elk. We compared concentrations of LH and progesterone of these females to those treated with leuprolide formulation (group A). Non-pregnant control females (group C) provided a more representative comparison to treated elk for evaluating treatment-induced hormonal responses than potentially pregnant elk, thus the need for two separate control groups.

### Treatments

**Leuprolide implant formulation.** The polymer, 85/15 poly (DL-lactide-co-glycolide) (PLG) with intrinsic viscosity 0.31 dL/g (Absorbable Polymer Technologies, Pelham, Alabama, USA) and N-methyl-2-pyrrolidone (NMP, International Speciality Products, Wayne, New Jersey, USA) were mixed in a ratio of 50:50 in a vial until the polymer was completely dissolved. The polymer solution was sterilized by  $\gamma$ -irradiation at a dose of approximately 25Gy (Isomedix, Morton Grove, Illinois, USA) and an appropriate amount of the sterilized polymer solution was filled into 1.2 luer-lock female syringes. For the leuprolide

part of the system, calculated volume of filtered aqueous solution of leuprolide acetate (Mallinkrodt, St. Louis, Missouri, USA) was filled in 1-mL male syringe barrels (Becton-Dickenson, Franklin Lakes, New Jersey, USA) and lyophilized. This formulation was designed to deliver a 32.5 mg dose of leuprolide at a controlled rate over a 180-day therapeutic period. A similar formulation was previously shown to suppress ovulation and pregnancy for one breeding season in captive elk when delivered subcutaneously by hand-injection (Baker et al., 2002).

***Treatment application.*** On the day before treatment application (6 September 2002), experimental elk were moved from holding paddocks to individual isolation pens (5 m x 10 m), weighed ( $\pm 0.5$  kg), sedated with xylazine hydrochloride (Rompun; Bayer AG, Leverkusen; 25-200 mg/animal, IM) and fitted nonsurgically with indwelling jugular catheters. The next day, and just prior to injection, separate syringes containing the polymer and the leuprolide were connected and the contents mixed with 60 back and forth mixing cycles. The resulting homogenous dispersion was drawn into the male syringe, and the formulation was transferred into single use, 1 ml, 13-mm-diameter, barb-less darts equipped with gel-collared 32-mm-long needles (Pneu-dart, Williamsport, Pennsylvania, USA). The final concentration of leuprolide was 12 % in the homogenous mixture of polymer solution and leuprolide acetate after mixing and was designed to deliver approximately 32.5 mg of leuprolide acetate to the elk. Control elk received only the polymer solution processed the same way but without leuprolide.

Prior to darting, individual elk were placed in a handling chute and lightly sedated with intravenous (IV) xylazine hydrochloride (15-20 mg/animal). This dose allowed animals to remain standing in the chute and minimized excitation associated with discharge of the dart gun. All elk were remotely injected with a dart fired from a CO<sub>2</sub>-powered pistol (DanInject™, Wildlife Pharmaceuticals, Fort Collins, Colorado, USA). In order to accurately determine the precise dose of leuprolide formulation delivered to each elk, darts were weighed before and after injection.

With the exception of two animals, one dart per animal was fired from approximately 3 meters into the area of the biceps femoris muscle of the standing elk. In two animals, the dart failed to discharge or only partially injected the prescribed dose. In these cases, we re-weighed and fired additional darts until the complete dose was delivered to each animal. Once all elk had been treated, sedation was reversed with yohimbine (30 mg, IV) (Antagonil®, Wildlife Laboratories, Fort Collins, Colorado, USA) and animals were returned to individual isolation pens.

## **Measurements**

*24 h LH response to leuprolide treatment.* Immediately following application of treatments to groups A and group C, we determined the amount of LH released during the initial 24 h of the treatment period. Blood samples (5 ml) were collected via jugular catheters at 0, 120, 180, 240, 300, 360, 480, 600, 960, and 1440 min after drug injection. Catheters were flushed after each collection with sterile saline solution. After the last blood collection, catheters were removed and animals were returned to holding paddocks. Eight days later, two intact male elk were placed into the same pasture with these females.

*Duration of LH and progesterone response to leuprolide treatment.* The effect of leuprolide formulation on the duration of suppression of LH and progesterone was determined by periodically conducting pituitary stimulation trials. These trials were performed prior to treatment application as an aid in the selection of animals for this experiment and periodically during 29 October 2002 to 3 September 2003 to determine pituitary responsiveness to an exogenous dose of GnRH analog (D-Ala<sup>6</sup>-GnRH-Pro<sup>9</sup>-ethylamide; Sigma Chemical Company, St. Louis, Missouri, USA).

Pituitary stimulation trials were conducted with elk in groups A and C elk at 50, 100, 150, 185, 215, and 361 days post-treatment. The final stimulation trial (3 September 2003) provided hormonal evidence of the reversibility of leuprolide treatment. Stimulation trials were conducted according to the

following procedures: On the day of testing, elk from groups A and C were moved from 5 ha pastures to individual isolation pens, weighed, sedated (as previously described), and fitted nonsurgically with indwelling jugular catheters. A bolus dose of GnRH analog (1 µg/50 kg body weight) was administered through the cannula and blood samples (5 ml) were collected at 0, 60, 120, 180, 240, 300, 360, and 480 min post-administration. After collections, blood was stored at 4 C for 24 h until serum was obtained by centrifugation (1500 RCF for 15 min). Serum for progesterone analysis was obtained from the 0 h blood sample for each animal on each of the trial days. Serum was stored at -20 C until analyzed for LH and progesterone. Following the last blood collection, catheters were removed, and elk were returned to holding pastures.

*Reproductive response to leuprolide treatment* - The effect of leuprolide formulation on reproduction in groups A and B was determined in two ways : (1) by measuring pregnancy rates using the presence or absence of pregnancy specific protein B (PSPB) (BioTracking, Moscow, Idaho, USA) in serum collected at approximately 100 and 215 days of gestation (Huang et al., 2000), and (2) by observing the presence or absence of calves the following summer.

### Analyses

Serum concentrations of LH were quantified by means of an ovine oLH radioimmunoassay (Niswender et al., 1969). Elk serum was demonstrated to inhibit binding of <sup>125</sup>I-labeled oLH to LH antiserum in a manner that paralleled the standard (NIH- oLH-S24). Similarly, when different quantities of oLH standard were added to elk serum and samples were subjected to radioimmunoassay, the values obtained were increased by the quantity of oLH added ( $r^2 = 0.99$ , slope = 0.92,  $\beta_1 = 0.22$ ,  $P = 0.002$ ). These data indicated that the radioimmunoassay provided a quantitative assessment of LH in elk serum. The limit of sensitivity of the LH assay was 0.02 ng /ml. Serum concentrations of progesterone were also determined by radioimmunoassay (Niswender, 1973). Sensitivity of the progesterone assay was 0.12 ng /ml. Intra-and-inter assay coefficients of variation for each of these assays were < 10 %.

Hormone concentrations are reported as untransformed arithmetic means ( $\pm$  SE). Responsiveness of the pituitary gland to GnRH analog stimulation was determined by the total amount of LH secreted (ng /ml/ min) which was estimated by calculating the area under the LH response curve (Abramowitz and Stegun, 1968). Differences among hormone concentrations were tested using least squares ANOVA for general linear models (SAS Institute, 1997). Responses to treatment were analyzed with one-way ANOVA for a randomized complete block design with repeated measures. Treatment effects were determined using the total animal-within-treatment variances as the error term. Time was treated as a within-subject effect, using a multivariate approach to repeated measures (Morrison et al., 1976). A “protected” least significant difference test (Milliken and Johnson, 1984) was used to separate means when the overall  $F$ - test indicated significant treatment effects ( $P < 0.05$ ).

## RESULTS

Intramuscular injection of leuprolide formulation via dart, was 100 % effective in suppressing ovulation and preventing pregnancy in captive female elk for one breeding season. All leuprolide - treated females (group A) tested negative and untreated controls (group B) positive for PSPB at approximately 100 and 215 days of gestation. No calves were born to treated elk, whereas the calving rate of untreated elk was 100 %. The amount of leuprolide acetate delivered to each elk ranged from 22.6 to 38.1 mg ( $\bar{x} = 33.1$ , SE = 2.4). We did not observe any unusual bleeding, swelling or trauma at the injection site nor did any of the elk show evidence of impaired mobility or post-treatment tissue necrosis or abscesses related to dart delivery of the bioimplant. Of particular interest was that the lowest individual dose delivered (22.6 mg) was equally as effective as higher doses in suppressing hormone concentrations

and pregnancy, suggesting that the minimum effective dose in elk could be substantially lower than the estimated dose (32.5 mg) used in this experiment.

Mean serum concentrations of LH increased ( $P = 0.015$ ) in treated elk (group A) within 2 h of drug injection, peaked at  $63.12 \pm 10.8$  ng/ml (mean  $\pm$  SE) 4.3  $\pm$  0.65 h (mean  $\pm$  SE) later, then gradually declined to baseline levels by 16 h post-treatment (Fig. 1). Levels of LH in group A were greater ( $P = 0.032$ ) than those of untreated controls (group C) for 2- 10 h post-treatment, after which, values decreased to baseline levels and were similar ( $P = 0.285$ ) for both groups.

Results of periodic GnRH challenges revealed that the leuprolide formulation reduced pituitary content of LH to basal concentrations for at least 215 days post-treatment, which was 35 days longer than the expected 180-day delivery period (Fig. 2). Concentrations of GnRH analog-induced LH secretion were lower ( $P = 0.022$ ) in leuprolide - treated elk (group A) compared to non-pregnant controls (group C) at days 50, 150, 185, and 215 days after treatment. Chronic suppression of LH in treated females was followed by a return to pretreatment levels, indicative of estrus, prior to the subsequent breeding season (September 2003, Fig. 3). In contrast to leuprolide-treated elk, pituitary responsiveness of untreated elk (group C) to GnRH analog were elevated and relatively similar ( $P = 0.64$ ) in magnitude during the first 185 days of the experiment, after which, these levels declined ( $P = 0.087$ ), presumably with the onset of seasonal anestrus (March). Similar ( $P = 0.582$ ) to treated elk, pituitary responsiveness in control elk (group C) returned to pretreatment levels in September 2003.

Serum concentrations of progesterone in leuprolide - treated females (group A) followed a parallel pattern to that observed for serum LH (Fig. 3). The suppressive effects of leuprolide on corpus luteum formation and steroidogenesis was readily apparent by its effects on serum progesterone concentrations in treated elk compared to controls (group C). Progesterone levels in treated elk declined ( $P = 0.017$ ) to limits of detection by 50 days post-treatment and remained at those levels for the duration of the breeding period. For untreated elk (group C), serum progesterone was more variable and consistently higher ( $P = 0.043$ ) than that for treated elk at 50, 100, 150, 185, and 215 days post-treatment. As evidence of normal estrous cycles and contraceptive reversibility, progesterone concentrations in both treated and untreated elk (group C) returned to pretreatment levels ( $P = 0.435$ ) at the onset of the following breeding season.

## DISCUSSION

In the present experiment, we evaluated the effectiveness of projectile dart delivery of the GnRH agonist, leuprolide, as a potential antifertility agent in female elk. The sustained release polymeric implant formulation of leuprolide acetate, remotely delivered in a projectile dart, resulted in decreased LH and progesterone secretion, presumably suppression of ovulation and steroidogenesis, and effective contraception (100 %) without adverse effects for one breeding season.

The contraceptive effects of leuprolide formulation followed a two-phase process. The first phase was characterized by an acute, transient rise in serum LH which gradually declined to basal concentrations about 16 h post-treatment. The second phase was defined by chronic inhibition of LH and progesterone secretion for the duration of the seasonal breeding period. Subsequently, normal ovarian function and fertility were re-established prior to next breeding season. We conclude from these patterns of LH and progesterone in serum that gonadotropes in female elk are down-regulated during treatment with GnRH agonist. As a consequence, long-term exposure to GnRH agonist resulted in reduction in GnRH receptors on gonadotropes (Clayton, 1989), depletion of pituitary LH and FSH content (Aspden et al., 1996), and elimination of the preovulatory LH surge (Gong et al., 1995; D'Occhio et al., 1996). These responses have been shown to result in ovulation failure and infertility which persists as long as the GnRH agonist is present in circulation at therapeutic levels (Melson et al., 1986; D'Occhio et al., 2000).



Our findings here are consistent with previous observations of acute and chronic responses of sheep (Dobson, 1985), cattle (D'Occhio et al., 1989; Gong et al., 1996), horses (Montovan et al., 1990), deer (Becker and Katz, 1995), and elk (Baker et al., 2002) treated with GnRH agonist.

Effective contraception in polyestrous, seasonal breeders is dependent on suppression of ovulation from the beginning of the breeding season to the onset of seasonal anestrus, a period of approximately 200 d in elk. Therefore, the timing of treatment application is an important consideration in successful contraception. Because of the acute rise in LH concentrations that occurs following GnRH agonist treatments, ovulation of growing follicles can be induced (Macmillan and Thatcher, 1991, D'Occhio and Aspden, 1999). Therefore, to ensure effective contraception in female elk, leuprolide treatments should be applied prior to the initiation of seasonal estrus.

In the present study, leuprolide inhibited LH secretion and ovulation for at least 215 days which is in close agreement with previous research, in which a subcutaneous dose of leuprolide suppressed LH levels for 190-250 days (Baker et al., 2002). In other studies, implants containing GnRH agonist have been shown to suppress ovarian activity for a minimum of 150 days in mule deer and (Baker et al., 2004) and almost 400 days in cattle (D'Occhio et al., 2002).

Persistent suppression of ovarian function, beyond the formulated delivery period of the implant, has been reported for a number of different species. Leuprolide suppressed LH and progesterone levels in elk in this experiment for at least 35 days longer (19 %) than the expected six month effective duration and 30 -110 days longer in deer and elk in previous studies (Baker et al., 2002, 2004). Similar observations of extended gonadotrope suppression were reported previously in cattle (Bergfeld et al., 1996; D'Occhio et al., 1996), monkeys (Fraser et al., 1987), men (Hall et al., 1999), and women (Broekmans et al., 1996). The underlying mechanism for this effect is not completely understood, but it is thought to be associated with prolonged dysfunction of gonadotrope cells rather than direct action on the ovaries (D'Occhio et al., 2000; Aspden et al., 2003). Regardless of the mechanism involved, the extended suppression of ovarian function, as a consequence of GnRH agonist treatment, is fundamentally essential to effective contraception in deer and elk.

In conclusion, intramuscular delivery of the sustained release biodegradable polymeric implant formulation of leuprolide via dart resulted in effective suppression of ovarian function and fertility in female elk for one breeding season with a return to normal reproductive function the following year. These results are particularly important for wildlife applications where, in the absence of such technology, animals must first be captured and restrained prior to treatment.

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Prepared by \_\_\_\_\_  
Dan L. Baker, Wildlife Researcher

Figure 1. Twenty-four hour serum LH concentrations (ng/ml, mean  $\pm$  SE) for untreated female elk (" , n = 5) and female elk (!, n = 6) treated with a 180-day sustained release implant formulation, containing approximately 32.5 mg of leuprolide acetate, remotely delivered via projectile dart.

Fig. 1 - Baker et al.

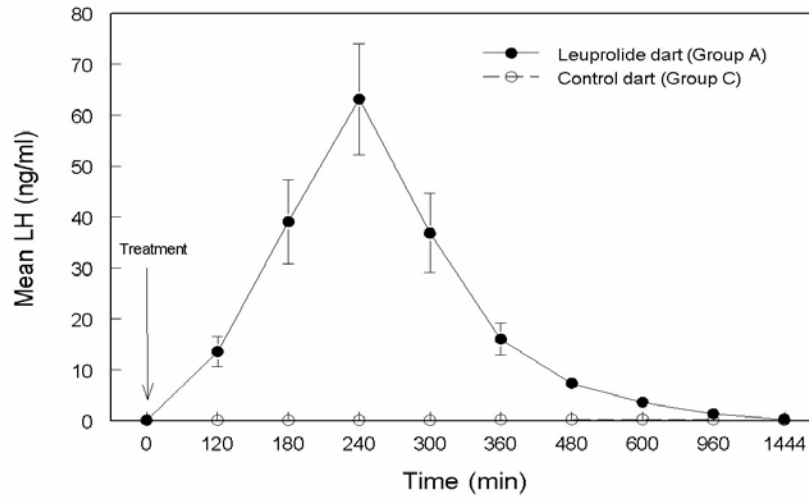


Figure 2. Total serum LH concentrations (ng/ml/min, mean  $\pm$  SE) for GnRH analog-induced release of LH for untreated female elk (" , n = 5), and female elk (!, n = 6) treated with a 180 -day sustained release implant formulation, containing approximately 32.5 mg of leuprolide acetate, remotely delivered via projectile dart. Different lower case letters indicate significant differences between means ( $P$  0.05).

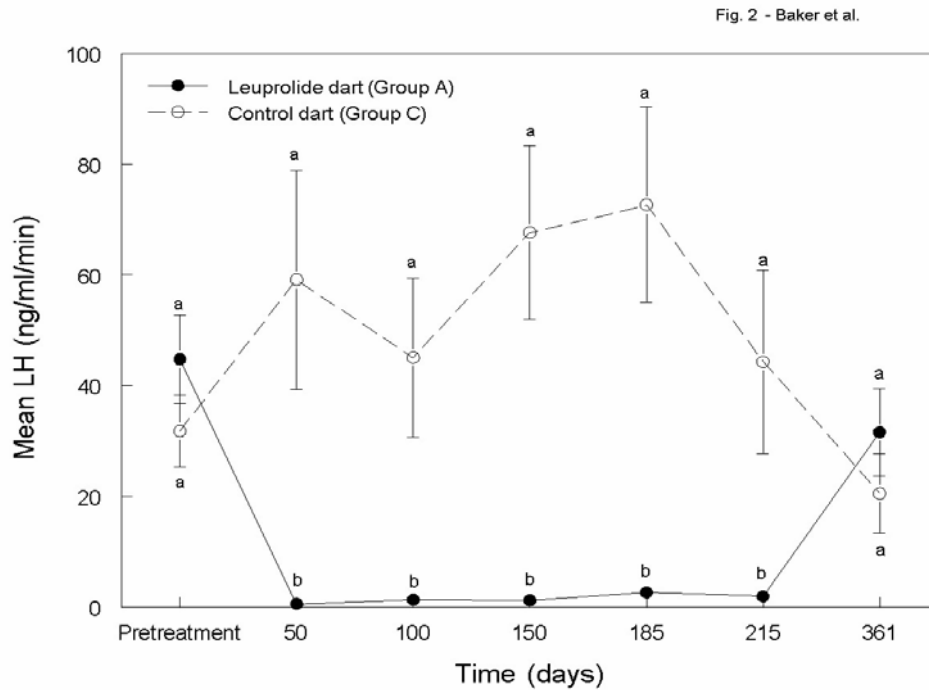
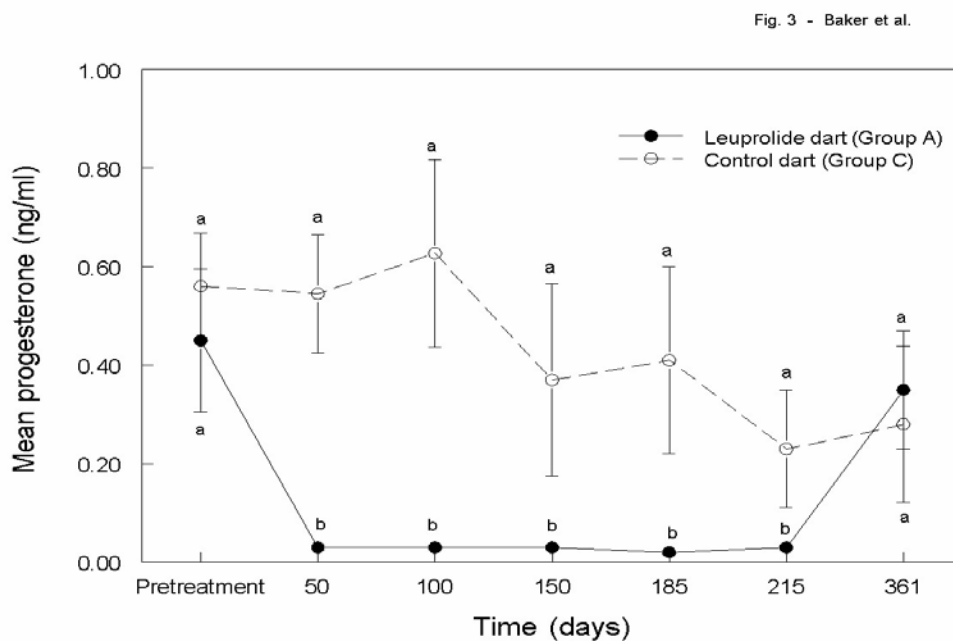


Figure 3. Serum profiles of mean progesterone concentrations (ng/ml, mean  $\pm$  SE) for untreated female elk (" , n = 5) and female elk (!, n = 6) treated with a 180-day sustained release implant formulation, containing approximately 32.5 mg of leuprolide acetate, remotely delivered via projectile dart. Different lower case letters indicate significant differences between means. ( $P$  0.05).





Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. \_\_\_\_\_ : Mammals Research  
Work Package No. 3002 : Elk Conservation  
Task No. 3 : Estimating Calf and Adult Survival Rates and  
Pregnancy Rates of Gunnison Basin Elk  
Federal Aid Project: N/A :

Period Covered: July 1, 2003- June 30, 2004

Author: D. J. Freddy

Personnel: D. Masden, R. Basagoitia, L. Spicer, B. Diamond of CDOW, Dr. G. C. White Colorado State University, and cooperators/contractors Gunnison Basin Habitat Partnership Program, M. Schuette of MountainScape Imaging, private land owners, and elk hunters.

**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 this segment, the transition of monitoring the remaining 119 radio-collared adult elk from research to management biologists was facilitated by providing databases, telemetry equipment, and other guidance as needed. Progress reports were completed, peer-reviewed publications on elk survival rates were initiated, and publications were accepted by peer-reviewed journals.

**JOB PROGRESS REPORT**  
**ESTIMATING CALF AND ADULT SURVIVAL AND PREGNANCY RATES OF GUNNISON**  
**BASIN ELK POPULATIONS**

DAVID J. FREDDY

**P. N. OBJECTIVE**

Estimate survival rates of calf, adult female, and adult male elk and estimate pregnancy rates of adult female elk in Gunnison Basin elk populations for 3 years. **NOTE:** Prioritization of available research funding resulted in discontinuing efforts to estimate calf survival, pregnancy rates and body condition during 2002-03 but allowed for monitoring adult elk survival through June 2003.

**SEGMENT OBJECTIVES**

1. Facilitate the transition of monitoring the remaining 119 radio-collared adult elk from research to management biologists by providing databases, telemetry equipment, and other guidance as needed.
2. Summarize and analyze data and publish information as Progress Reports, peer-reviewed manuscripts for appropriate scientific journals, or Colorado Division of Wildlife (CDOW) technical publications.

**SUMMARY**

Progress reports were completed for the Gunnison Basin elk project (Freddy 2002, Freddy 2003) and can be obtained through the CDOW Research Center library in Fort Collins, Colorado.

Publications incorporating calf and adult elk survival rates measured in the Gunnison Basin and Grand Mesa, Colorado were initiated.

Two publications were accepted by the Wildlife Society Bulletin for publication during this segment with authors and abstracts provided here for reference.

**How many mule deer are there? Challenges of credibility in Colorado**

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**Abstract:** Conflict resolution between stakeholder groups and management agencies is a problem in wildlife management. We evaluated our success in resolving a conflict between sportsmen and the Colorado Division of Wildlife (CDOW). Sportsmen challenged the credibility of methods used to estimate numbers of mule deer (*Odocoileus hemionus*) in Colorado and demanded validating surveys to verify numbers of deer. Sportsmen, other interested wildlife stakeholders, and CDOW engaged in a conflict resolution process and designed and implemented an aerial survey to estimate numbers of deer in a specific population whose previous estimated size had been contested by sportsmen. We used helicopters to count mule deer on randomly selected sample units distributed on deer winter range in March 2001. Estimated population size was  $6,782 \pm 2,497$  (90% CL) using stratified random sample estimators and  $11,052 \pm 3,503$  (90% CL) when counts of deer were adjusted using the Idaho mule deer sightability model. Both aerial survey estimates supported computer modeled population estimates of 7,000 to 7,300 deer that had been contested by sportsmen and all estimates were greater than the sportsmen's estimate of 1,750 deer which was determined from their casual observations. After the survey, sportsmen did not accept survey estimates despite their involvement in design, analysis, and interpretation of the validation survey. By failing to support results of a validation survey they had demanded, the credibility of sportsmen plummeted among other stakeholders, the Colorado Wildlife Commission, and outside public entities while credibility of CDOW managers rose. We contend that CDOW successfully met challenges of sportsmen because the aerial survey systems used to validate deer numbers were founded on credible science and applied within a resolution process that elicited trust from most stakeholders. We caution other agencies facing similar challenges to use tested methods that can withstand public scrutiny, allow ample time for planning and implementing, carefully assess technical and political risks associated with potential outcomes, and engage multiple stakeholders in planning efforts to gain trust of participants. Cost of this resolution process was about 100,000 \$US.

**Key words:** Colorado, conflict resolution, credibility, helicopter surveys, human dimensions, mule deer, *Odocoileus hemionus*, population estimates, sightability

*Wildlife Society Bulletin* 32 (3):00-00.

### **Effect of limited antlered harvest on mule deer sex and age ratios**

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**Abstract:** During the 1990s, in response to apparent declining mule deer (*Odocoileus hemionus*) numbers in Colorado, high buck harvest rates were identified as one of several factors that could be negatively affecting population productivity. Some wildlife managers and sportsmen hypothesized that increasing buck:doe ratios by limiting buck harvest would cause an increase in fawn:doe ratios, and hence, population productivity. We evaluated this hypothesis using data collected by the Colorado Division of Wildlife (CDOW) from 1983 to 1998. Beginning in 1991, CDOW reduced buck harvest in 4 deer management units to provide quality hunting opportunities while maintaining high harvests in other management units. We examined effects of limited harvest on December ratios of bucks:100 does and fawns:100 does using data obtained from helicopter surveys in limited and unlimited harvest units.

Annual buck harvest was reduced by 359 bucks (SE = 133) as a result of limiting licenses in the 4 limited harvest units. Fawn:doe ratios declined by 7.51 fawns:100 does (SE = 2.50), total buck:doe ratios increased by 4.52 bucks:100 does (SE = 1.40), and adult buck:doe ratios increased by 3.37 bucks:100 does (SE = 1.04) in response to limited harvest. Evidence suggested that factors other than buck harvest were regulating population productivity with density dependence being a plausible explanation of declining fawn:doe ratios. Limiting buck harvest to enhance fawn recruitment is not justified in Colorado based on our analysis. Management for limited buck harvest should be largely framed as an issue of quality hunting opportunity rather than an issue of deer productivity.

**Key words:** age ratio, buck:doe ratio, Colorado, fawn:doe ratio, limited harvest, mule deer, *Odocoileus hemionus*, productivity, quality hunting, sex ratio

**Wildlife Society Bulletin 33 (0):00-00.**

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Prepared by \_\_\_\_\_  
David J. Freddy, Wildlife Researcher

Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. 1 : Mammals Research  
Work Package No. 3003 : Predatory Mammals Conservation  
Task No. 1 : Colorado Puma Research & Management  
Program  
Federal Aid Project: N/A :

Period Covered: July 1, 2003— June 30, 2004

Author: K. A. Logan

Personnel: J. Apker, J. Kindler, Colorado Division of Wildlife; L. Mundy-Four Corners Houndsmen's Association; and Safari Club International 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

The Colorado Puma Research and Management Program started in July 2003. The goal is to improve the scientific foundation of puma management by the Colorado Division of Wildlife. The program was developed with inputs of Division researchers, managers, and biologists, and Colorado citizens interested in wildlife management, hunting, and the environment. Puma population research is scheduled to begin in November 2004. Associated projects to improve puma management in Colorado, also initiated this year include: the Colorado puma data map, prospective work for Front Range puma-human interaction research, puma technical workshops, and Data Analysis Unit management plans and puma-human conflict guidelines.

**JOB PROGRESS REPORT**  
**COLORADO PUMA RESEARCH AND MANAGEMENT PROGRAM**

Kenneth A. Logan

**INTRODUCTION**

The Colorado Puma Research and Management Program started in July 2003. The goal is to improve the scientific foundation of puma management in Colorado. A Prospectus was developed with inputs of Division researchers, managers, and biologists, and Colorado citizens interested in wildlife management, hunting, and the environment. The major part of the program is the puma research project in the prospectus, scheduled to begin in November 2004. The initial design of the research will be clarified in a study plan in September 2004 which will pertain to the puma population research on the Uncompahgre Plateau study area. Other work associated with the development of the research program included: visiting with affected publics (private landowners, ranchers, hunters, guides and outfitters) and agency cooperators, surveying potential study areas, and two public meetings for information on the proposed puma research. Associated projects to improve puma management in Colorado included: the Colorado puma data map, puma workshops, technical advice on puma Data Analysis Unit management plans, and puma-human conflict guidelines.

**COLORADO PUMA DATA MAP**

The objective of this project is to map and quantify puma data that exists in records of the Colorado Division of Wildlife. This is the first step for Division staff to examine historical and current situations regarding puma management actions and puma mortality patterns state-wide and within management units. The map is intended to be an evolving instrument that allows comparisons with puma data gathered in the future to examine potential effects of changing puma management prescriptions, habitat, ungulate populations, and human developments. Interpretations of the map could be clarified from information on puma populations, movement patterns, habitat use, habitat characteristics, puma-ungulate interactions, and hunter access to occupied puma habitat.

Reliable interpretations of such maps would be useful to managers. Number and distribution of puma mortality locations and absence of mortality locations may indicate relative puma abundance or hunting pressure. High mortality areas, influenced principally by sport-hunting pressure, may indicate potential areas of high puma densities, puma population sinks (defined as areas where the average population growth rate is negative), areas of facilitated puma hunting conditions (e.g., high road density, consistent snow coverage), and liberal puma harvest objectives. Low puma mortality areas and blank areas on the map may indicate potential puma habitat with puma source populations (defined as areas where mean population growth rate is positive, and which serve as net exporters of dispersing animals), areas where few if any puma live, or areas with low hunter access or good hunting conditions.

**RESULTS**

Desired products are maps and associated tabulated data on geographical distribution and intensity of puma mortality. Puma mortality data (including sport-harvest, depredation control, public safety management, and accidental deaths) recorded by the Division on mandatory check forms since 1997 were mapped, state-wide and by Data Analysis Units and Game Management Units, and stratified by year and puma sex and age class (e.g., adult, subadult, cub). This entails about 2,423 data points state-wide, from 1997—2002 (2003 data have not been entered, yet). Of that, over 90% of the mortality locations are due to sport-harvest. The remainder is due to depredation or public safety control kills, road-kills, and other recorded deaths. Maps are currently in a preliminary development stage. Mortality

locations of puma will be buffered by average puma home range sizes for adult puma in western North America (195 km<sup>2</sup> for females; 357 km<sup>2</sup> for males) and overlaid by mule deer, elk, and bighorn sheep winter ranges.

In addition, the identity of DAUs with the management objective of a stable or increasing puma population and DAUs with the management objective of a suppressed population also need to be mapped for managers to consider the number, distribution and effects of potential source and sink populations. Other map overlays that may facilitate interpretation of the puma data include: road distribution (i.e., paved, all-weather, dirt), vegetation cover types, elevation, and human developments and density. Puma mortality characteristics (i.e., location, density) might be modeled by using an analytical approach that uses habitat and biological features (e.g., ungulate distribution and relative density, elevation, roads, vegetation, terrain ruggedness) as variables. Another approach might be to distribute puma mortality maps to Division field personnel and to knowledgeable puma hunters to record their explanations about geographical puma distributions, relative densities, mortality patterns, and effects of habitat characteristics (e.g., landownership, snow conditions, access).

## **COLORADO PUMA-HUMAN INTERACTIONS**

### **RESULTS**

Meetings involving Division staff, and individuals from the U.S. Geological Survey Ft. Collins Research Center and Colorado State University were held to discuss potentials for puma-human interaction research on the Colorado Front Range. Meetings were held in conjunction with field trips to explore potential study areas west Ft. Collins on October 6, 2003; west of Boulder on January 28 and February 6, 2004; and west of Colorado Springs on February 5, 2004. Division staff from the southeast and northeast expressed a great deal of interest in developing puma-human interaction of research in the next 1—2 years, and discussions indicated the need to develop a reliable funding base and connections with potential cooperators.

The Division of Wildlife and Four Corners Houndsmen's Association co-hosted four workshops in 2004 to inform hunters and other interested citizens about puma in Colorado. Workshops were held in Grand Junction (July 19), Alamosa (July 17), Denver (July 22), and Canon City (August 14). In all, the workshops were attended by about 60 people. Workshop agenda topics included: puma population characteristics, vital rates, reproductive biology, behavior, prey selection, female and cub vulnerability to hunting, gender identification in the field, aging techniques, Colorado puma data map, puma management, Data Analysis Unit plans, quota setting process, proposed puma research, and puma-human conflict management. A PowerPoint presentation was developed as the main source of information on these topics. Some attendees suggested that such workshops should be held periodically for puma hunters and other people interested in puma.

Associated with this effort to bring reliable information on puma to hunters, we also produced printed guidelines for sexing puma in the wild. This information is available on the Division's webpage: <http://wildlife.state.co.us/hunt/BigGame/pdf/MtLionGender.pdf> and in APPENDIX I of this progress report.

Drafts of guidelines developed by Division managers for addressing incidents when puma conflict with people were discussed and reviewed and a final draft was created for review by Division staff: Draft—Human Mountain-Lion Incidents.

## **PUMA RESEARCH AND MANAGEMENT PROSPECTUS PROBLEM STATEMENT**

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 Division objective of actively managing puma while “achieving healthy, self-sustaining populations”(Colorado Div. of Wildlife 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.

Members of the Division’s Mammals Research staff met with Division wildlife managers and biologists from the Northwest, Southwest, Southeast, and Northeast Regions regarding puma management issues and the resultant research needs. In addition, we consulted with other agencies, organizations, and interested publics either directly or through other Division employees. In general, Division staff in western Colorado conveyed concern about puma population dynamics, especially as they relate to their abilities to manage puma populations through regulated sport-hunting. Secondly, (perhaps because of results from recent research findings in western Colorado), they expressed interest in puma-prey interactions. Division managers on the Rocky Mountain’s 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. Specific management needs and lines of inquiry identified by Division staff and public stakeholders are categorized as follows:

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).
- 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 puma.
- Effects of aversive conditioning on puma.

Past Puma Research in Colorado

Data from past puma research in Colorado that address the topics above are limited. Currier et al. (1977:8) studied 29 captured puma on 2 tracts— one 900 km<sup>2</sup>, and one 1,950 km<sup>2</sup>— in Fremont and Custer Counties during 1974—1977. The puma population under study was subject to sport-hunting. Hunters killed a total of 31 puma in 3 winters. Non-systematic puma track counts were used to estimate

the minimum puma population at 11 on the 900 km<sup>2</sup> tract (density = 1.2/100 km<sup>2</sup>) and 25—28 on the 1,950 km<sup>2</sup> tract (density = 1.3—1.4/100 km<sup>2</sup>). The Petersen mark-recapture method was used to estimate 95 puma (95% CI = 35, 155; density = 4.8/100 km<sup>2</sup>) during 1977—1978; however, researchers probably did not meet 4 of the 6 assumptions needed for valid estimates of puma numbers (Anderson 1983:61, 63). Vital rate data were limited to mean litter size of 2.1 (range = 1—5, n = 14).

An effort to estimate puma population density in Game Management Units (GMU) 33 (Garfield and Rio Blanco Counties) and 40 (Mesa County) was made during 1980—1983 (Brent 1981, 1982, 1983). A total of 38 puma were captured: 21 were marked and released; 8 were released unmarked; 9 were killed for livestock depredation control (8) or during handling (1). Twelve puma were captured in GMU 33 and 26 were captured in GMU 40. Crude adult puma density estimates for GMU 33 ranged from 2.7—3.1 puma/100 km<sup>2</sup>. GMU 40 crude adult puma density estimates ranged from 1.2—3.7 puma/100 km<sup>2</sup>.

Anderson et al. (1992) studied 57 captured puma on 3,426 km<sup>2</sup> of the eastern slope of Uncompahgre Plateau in Mesa, Delta, Montrose, and Ouray counties during 1981—1988. Puma density was estimated only for 1987; the minimum density (mean ± SE) of residents was 1.1 ± 0.15 pumas/100 km<sup>2</sup>. Male to female sex ratios for 26 captured puma 1—12 months old was 1:1; for 19 captured puma ≥24 months old, it was 1:1.4. Age structure in that sample was 66.7% <24 months old and 33.3% ≥24 months old (the class most likely comprised of breeding adults). Vital rates included, mean (± SD) litter size of 2.4 ± 0.80 (n = 17), birth interval of 12 months (n = 2 intervals for 1 female), estimated annual survival rate for 42 puma of both sexes of 88.0 % (90% CI = 83.1, 91.4). Humans caused 18 of 21 deaths in radio-collared puma even though the study population was supposed to be protected from human off-take. Anderson et al. (1992) examined aerial locations of 7 radio-collared puma and subjective estimates of relative deer and elk density categories and could not identify consistent relationships probably because of the small non-random sample of puma, the subjective nature of the ungulate density categories, and other non-quantified factors. Mean annual home range sizes ranged from 436—732 km<sup>2</sup> for 3 males and 190—463 km<sup>2</sup> for 7 females. All of 9 radio-collared subadult male puma dispersed from natal areas. Two of 6 radio-collared subadult females did not disperse. Means and extremes of dispersal distances were 86.2 km (23—151) for 8 males that were 10—13 months old and 37.0 km (17—54) for 4 females that were 11—31 months old. Data on puma—human interactions were from 17 responses to 40 questionnaires submitted to residents in the housing development on the southeastern extreme of the study area. Seven of 17 respondents reported 25 puma sightings during about 260 months of residence. Ten respondents did not observe puma in about 476 months of residence.

Koloski (2002) studied 19 captured puma on the 2,758 km<sup>2</sup> Southern Ute Indian Reservation in La Plata, Archuleta, and Montezuma Counties during 1999—2001. The puma population was not subject to sport-hunting at the time. Transect intercept probability sampling was used in 2001 to estimate the puma population at 55 (90% CI = 9.0, 114.4) and a density of independent puma at 2.7/100 km<sup>2</sup>. Male to female ratio of the captured sample of 14 independent puma was 1:2.8. Of 16 captured pumas that were aged, 31% were <24 months old and 69% ≥24 months old. Vital rates included: litter size (mean ± SD) of 2.5 ± 0.58 (n = 4), birth interval of 16 months (n = 1), annual survival rate for radio-collared males (mean ± SD) of 0.89 ± 0.19 (n = 3) and radio-collared females of 0.72 ± 0.19 (n = 8); earliest age for female reproduction at 2—3 years; annual reproductive rate for resident females (mean ± SD) of 42% ± 12%. Mean home range sizes were 252.4 km<sup>2</sup> for 3 radio-collared males and 182.4 km<sup>2</sup> for 8 radio-collared females. Road density within puma home ranges and core areas was lower than that on the landscape where pumas occurred (P ≤ 0.002). Distance from puma locations to nearest roads was lower than distance from random points to nearest roads (P = 0.002).

## Current Puma Research in Colorado

Presently, researchers with the Colorado Division of Wildlife (Mike Miller, Ph.D., DVM) and Colorado State University (Caroline Krumm, Graduate Student and Dr. N. Thompson Hobbs, Advisor) are conducting puma research in Larimer County. The research goal is to test for selective puma predation on mule deer infected with chronic wasting disease (CWD) by comparing CWD prevalence in puma-killed deer to prevalence in harvested deer. The research protocol calls for 6 or more puma fitted with global positioning system (GPS) collars.

## RESEARCHABLE OBJECTIVES

The management issues listed previously in the PROBLEM STATEMENT may be translated into a number of researchable objectives, requiring descriptive studies and field experiments (Fig 1). Our goal is to provide managers with reliable information on puma biology and ecology and to develop and test tools for their efforts to adaptively manage puma in Colorado to maintain healthy, self-sustaining populations.

Researchable objectives address managers' main needs. We propose that the Division begin to address objectives that focus on puma population dynamics, effects of harvest, and estimating puma population abundance with an intensive puma population study on the West Slope. Those objectives include:

1. Describe and quantify puma population characteristics, including: density, sex and age structure.
2. Describe and quantify puma population vital rates, including: birth rates, age or stage-specific survival rates, emigration rates, immigration rates.
3. Describe and quantify agent-specific mortality rates and vulnerability of different classes of puma to hunter harvest and quantify agent-specific mortality rates.
4. Develop and test puma population models using metrics from objectives 1—3.
5. Develop and test indices to puma abundance calibrated on an estimated puma population (i.e., puma track counts, catch per unit effort, DNA genotyping).

In addition, other objectives could be partially addressed during the intensive puma population research effort (i.e., objectives 1—5). Those include:

6. Describe and quantify relationships of puma to people and human facilities on the study area.
7. Describe and quantify puma use of habitats and landscape linkages.
8. Describe and quantify relationships of puma to mule deer, elk, and other prey.
9. Describe and quantify responses of puma to aversive conditioning.
10. Describe and quantify behavior and survival of translocated puma.

Data collection for primary objectives 1—5 will often have applicability to objectives 6—10. For example, GPS-collared puma will enable us to quantify puma predation rates on ungulate prey, puma use of habitats and landscape linkages, puma-human interactions, and behavior and survival of translocated puma (if puma are removed from a study area as part of an experimental manipulation). However, we cautioned that such opportunistic data gathering likely will not yield the power or confidence levels of studies specifically designed to meet those objectives. Yet, such efforts could function as pilot studies. Additional research efforts can be phased in later in the puma research program. And some, (e.g., puma relationships to people, puma use of habitats and landscape linkages) can be conducted in different areas of Colorado.



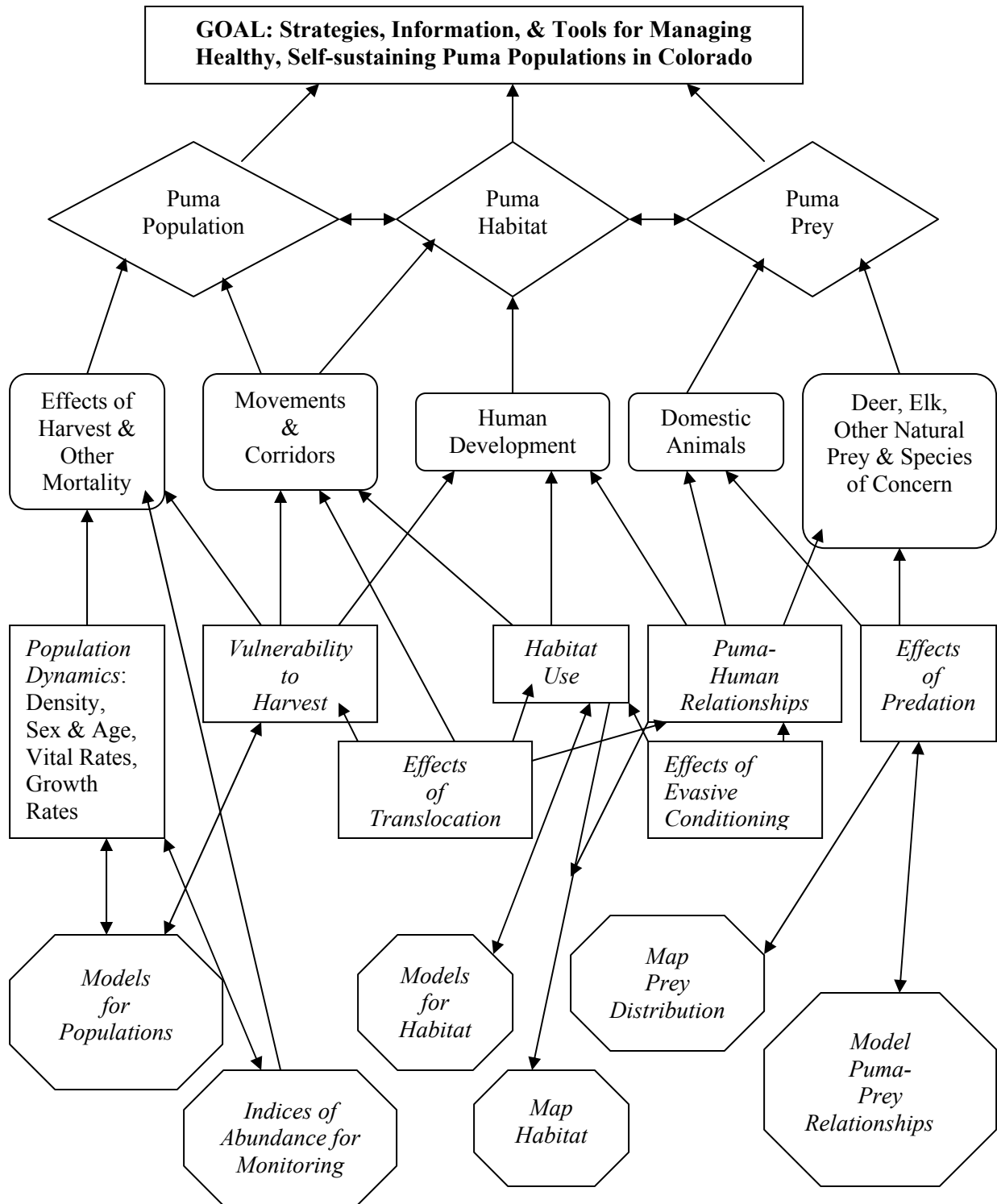


Fig. 1. Conceptual model of the Colorado Puma Research & Management Program.

## TESTING ASSUMPTIONS AND HYPOTHESES

Hypotheses associated with the main objectives can be structured to test assumptions, information, and methods that may guide puma management in Colorado.

1. Lacking Colorado-specific information, managers might assume that puma population densities in Colorado are within the range of those quantified in other populations studied in Wyoming (Logan et al. 1986), Idaho (Seidensticker et al. 1973), Alberta (Ross and Jalkotzy 1992), and New Mexico (Logan and Sweanor 2001). The Division has used density ranges of 2.0—4.6 puma/100 km<sup>2</sup> to extrapolate to Data Analysis Units to estimate a range of 3,000—7,000 puma in Colorado and to guide the quota-setting process. Likewise, managers may assume that the population sex and age structure is similar to puma populations described in the above-mentioned studies. Using capture, mark-recapture (or resight) techniques, a descriptive study will test H<sub>1a</sub>: The puma population density on the study area will vary within the range of 2.0—4.6 puma/100 km<sup>2</sup> and will exhibit a similar sex and age structure to puma populations in Wyoming, Idaho, Alberta, and New Mexico.

Yet, an experimental study that allows puma population growth to approach carrying capacity in high quality puma habitat can test if a puma population in Colorado might exceed published density estimates. H<sub>1b</sub>: Puma population density in high quality puma habitat in Colorado exceeds the high range of 4.6 puma/100 km<sup>2</sup>.

2. Background material that guides puma management in Colorado assumes a moderate rate of growth of 15% for the adult puma population. Theoretically, consideration of management changes would occur if hunter kill exceeds 15% of the low end adult puma population estimate. A field experiment, involving an increase population growth phase, is required to test H<sub>2</sub>: The estimated average annual adult puma population growth rate in high quality puma habitat in Colorado (during an increase phase) will match or exceed the hypothetical  $r = 0.15$ .
3. The same background material assumes “that when female” puma “comprise greater than 50% of the hunter harvest it is an indicator that hunting may be acting to suppress the population.” An experimental study with a decline puma population growth phase will test H<sub>3</sub>: The population of harvest-age puma (i.e., adults, subadults) will decline only when 50% or more of the harvest is comprised of harvest-age female puma (i.e., independent subadults  $\approx \geq 12$ —24 mo. old and adults  $\geq 24$  mo. old).
4. Colorado puma Data Analysis Units (DAUs) or other management units may behave as a demographic source-sink metapopulation structure where the puma population of a region is comprised of subpopulations each of which may have dynamics that are not necessarily correlated with other subpopulations. Source-sink metapopulation dynamics function as a result of variable puma habitat quality and management practices (e.g., prey population dynamics, harvest pressure). Sources are increasing or stable populations where recruitment via local reproduction and immigration equal or outpace mortality. These source populations produce emigrating progeny that immigrate into other subpopulations, augmenting them numerically and genetically. Comparatively, sink populations are those where mortality exceeds recruitment, and puma numbers are declining or suppressed to a relatively low density. Sink populations contribute few emigrating progeny as potential recruits for other subpopulations. Sink populations are augmented by immigrants from source populations (Sweanor et al. 2000, Logan and Sweanor 2001). This project will examine H<sub>4</sub>: The study population will exhibit characteristics of a subpopulation in a demographic source-sink metapopulation structure. The following predictions must come true to support this hypothesis.

- a. The majority (i.e., >50%) of male recruits in the adult segment of the population on the study area will be immigrants (Logan and Sweanor 2001). Immigrants will not be offspring of puma on the study area as determined by genetic parentage analysis.
  - b. Up to one-third of female recruits will be immigrants (Logan and Sweanor 2001).
  - c. Male and female immigrant recruits will produce viable young.
  - d. The majority of male progeny from the study area will emigrate (Logan and Sweanor 2001).
  - e. About one-third of female progeny from the study area will emigrate (Logan and Sweanor 2001).
  - f. Movements of male and female emigrants will be large enough to carry them to other Data Analysis Units with differing management objectives (i.e., population reduction, population stability).
  - g. Male and female emigrants will establish adult home ranges in other puma habitats in Colorado. H<sub>4A</sub>: Recruits born in the local population are the largest contributor (i.e., >80%) to the maintenance of the puma population on the study area.
5. In southern Utah, Van Sickle and Lindzey (1992) found a positive relationship ( $r^2 = 0.73$ ) between the number of radio-collared puma known to have home ranges overlapping dirt roads (response variable) and track-finding frequency (explanatory variable). Similarly, researchers in Montana are finding a positive relationship between the number of puma home ranges overlapping search routes and puma track density (DeSimone et al. 2002). This relationship should reflect changes in puma numbers on the survey area, and thus may be useful as an index to relative abundance. A field experiment requiring both increase and decrease puma population growth phases is required to test H<sub>5a</sub>: Puma track-finding frequency (response variable) is positively correlated to number of puma with home ranges overlapping snow-covered search routes (explanatory variable). H<sub>5b</sub>: Puma track-finding frequency (response variable) is positively correlated to the density of independent puma (explanatory variable).
  6. Theoretically, the amount of effort (i.e., hunting days) that hunters spend in pursuit of finding harvest-age puma (i.e., adults and subadults) should be proportionate to the abundance of puma (Lancia et al. 1996). A relationship should exist between changes in catch (or encounter)-per-unit-effort and population changes. A field experiment, involving manipulation of the study population (i.e., increase phase, decline phase), is required to test H<sub>6a</sub>: Catch-per-unit effort of the research team in the increase phase, teams in the capture-recapture occasions during the increase and decline phases, and puma hunters during the decline phase will reflect the trend in the puma population. There will be an inverse (i.e., negative) correlation between the mean number of days per capture (response variable) and the number of independent puma in the population (explanatory variable) during the increase phase and the decline phase. H<sub>6b</sub>: During the increase phase, there will be a positive correlation between the mean number of days per capture of unmarked puma (response variable) and the number of marked puma “removed” from the unmarked population per year (explanatory variable). In the decline phase, there will be an inverse correlation between the mean number of days per capture (response variable) and the number of puma killed by hunters per year (explanatory variable).
  7. Relative vulnerability of puma to hunters is limited to information from 2 studies on the same area in southern Utah (Van Dyke et al. 1986, Barnhurst 1986). Van Dyke et al. (1986) quantified effort to locate 4 classes of puma by looking for their tracks on dirt roads, a method that hunters use to find puma. He found that cubs and adult females required the least effort, followed by independent subadults and adult males. In contrast, Barnhurst (1986) assessed vulnerability based on the relative road crossing frequencies of radio-collared puma in each of 7 classes that were relocated once per week. He found that the most vulnerable puma were subadult males, followed

by adult resident males, subadult females, and adult females (in 4 classes— females with 0 cubs, females with cubs 0-6 mo. old, females with cubs 7-12 mo. old, females with cubs 13-18 mo. old). Mothers with 0—6 month-old cubs had the lowest road-crossing frequency of all classes. Cubs in this age class are most vulnerable to death if their mothers die (K. Logan, unpublished data). A descriptive study will test  $H_7$ : Relative vulnerability of GPS-collared puma on the study area, based on road-crossing frequency per day, will reflect the results of Van Dyke et al. (1986).  $H_{7A}$ : Relative vulnerability of GPS-collared puma on the study area, based on road-crossing frequency per day, will reflect results of Barnhurst (1986).

8. Studies on the effectiveness of puma translocation, and the behavior, survival, and agent-specific mortality of translocated puma in western North America are limited to 2 studies. Ruth et al. (1998) reported on 14 puma translocated from 338—510 km in New Mexico, and Ross and Jalkotzy (1995) reported on 3 puma that were translocated 51—94 km in Alberta. The New Mexico research found that translocation was most successful for puma that were 12—27 months old, the age at which puma naturally attempt to disperse and search for a home range or establish a home range if they are philopatric. Older adult puma attempt to move back to their original home ranges. They found that mortality rates for translocated puma were significantly higher than mortality rates of non-translocated puma in a reference population. If translocation is used to experimentally reduce the population, this research would test  $H_8$ : Translocation of puma will exhibit similar characteristics to the New Mexico results. For this hypothesis to be supported, the following predictions must be true.
  - a. Mortality rates of translocated puma will be significantly higher than mortality rates of non-translocated puma.
  - b. Independent puma 12 to about 30 months old will establish home ranges in or near release areas and have relatively greater survival rates than older adult translocated puma.
  - c. Adult puma about 3 years old and older will tend to move back toward their original home ranges.

### **DESIRED OUTCOMES AND MANAGEMENT APPLICATIONS**

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 model(s) useful for estimates of puma population abundance and trends, evaluation of management alternatives, and effects of management prescriptions.
2. Indices to puma abundance or trends of known reliability will allow managers to “ground truth” modeled populations and estimate effects of management prescriptions designed to achieve specified puma population objectives.
3. Testing assumptions about puma populations, currently used by Division managers, will help those managers adapt puma management based on Colorado-specific estimated characteristics and dynamics of puma populations.
4. An understanding of relative vulnerability of the various puma sex and age classes to harvest could enable managers to better structure harvest data collection and interpretation, and to develop novel prescriptions to meet management objectives.
5. Functional relationships between population vital rates and population density could be examined. Puma life history traits in Colorado may be used to test generalized hypotheses regarding puma life history strategies in the literature, and inform managers to structure successful management strategies.

6. Determining whether or not the puma population in Colorado has a source-sink demographic source-sink structure is important to evaluating the current Game Management Unit and Data Analysis Unit structure of puma management and potential effects of the juxtaposition of puma sub-populations in Colorado managed for stability, suppression, or that may function as refugia.
7. Knowledge of the relationships of puma to deer, elk, and species of special concern would allow managers to realistically consider potential effects of puma predation on those prey in the development of management strategies and policy. In addition, such information would enable the testing of scientific hypotheses on relationships of puma to their prey.
8. In the study areas currently being contemplated, some puma home ranges will probably contain human habitations and other facilities. GPS-collared and VHF-collared puma will generate quantitative information on puma behavior in relation to human activity and assist managers to better inform people about ways of reducing potential conflicts between people and puma, and to structure puma conflict policy.
9. Habitat use data gathered during the course of this research could be used to quantify puma habitat characteristics on the study area, as well as habitats and landscape linkages used by dispersing puma. Such information could be used to structure more extensive investigations of puma habitat that contribute to habitat modeling efforts that may help identify puma habitat in Colorado. This would allow a more realistic conceptual inventory of puma habitat in the state.
10. This information could be disseminated to public stakeholders interested in pumas in Colorado, and thus contribute to informed public participation in puma management.

## **STUDY AREAS**

Three potential study areas were evaluated and are under consideration: the Plateau Creek-to-South Canyon area (in Garfield, Mesa, Gunnison, and Pitkin counties), the lower Dolores River-to-Disappointment Creek area (in Dolores and Montezuma counties), the South Uncompahgre Plateau (in Mesa, Montrose, Ouray, and San Miguel Counties) (Table 1). These areas appear to have attributes conducive to an intensive puma research effort, including sufficient area ( $400\text{--}500\text{ mi.}^2 = 1,036\text{--}1,295\text{ km}^2$ ) of puma habitat and suitable road access. Preferably, there should be a  $300\text{--}400\text{ mi.}^2$  buffer zone around the study area to reduce the effect of puma harvest.

Table 1. Potential puma study area locations and characteristics.

Location	Area	Other Attributes
Dolores River-to-Disappointment Creek (GMUs 71 & 711)	~840 mi. <sup>2</sup> = 2,176 km <sup>2</sup>	Large enough for core study area and buffer. Ratio of public:private land (mi. <sup>2</sup> ) ~4:1. Town of Dolores is at the south end of this area. Substantial number of people live on the plateau. Domestic sheep and cattle use the area. Puma hunting pressure is moderate. Puma predation on domestic animals is low.
Junction of Interstate 70 & State Route 6 northeast to South Canyon (GMU 42)	~400 mi. <sup>2</sup> = 1,036 km <sup>2</sup> Area can be expanded ~155 mi. <sup>2</sup> (~401 km <sup>2</sup> ) by adding northern portion of GMU 421 (north of state routes 6 & 330, the north slope of the Plateau Creek drainage).	Minimum study area size. Ratio of public:private land (mi. <sup>2</sup> ) ~2:1. Substantial number of people live along the I-70 corridor and in lower Mamm, Hollow, Divide, and Battlement Creeks, and on Grass Mesa. Recent research on elk seasonal movements, survival and cause-specific mortality rates (Freddy). An unknown number of cattle and horses use the area, but there are no domestic sheep. Gas exploration and development is occurring on the area. Puma hunting pressure is moderate. Puma predation on domestic animals is low.
South Uncompahgre Plateau (southern halves of GMUs 61 & 62)	~870 mi. <sup>2</sup> = 2,253 km <sup>2</sup>	Large enough for core study area and buffer. Ratio of public:private land (mi. <sup>2</sup> ) ~3:1. Ongoing mule deer research (Bishop et al. 2003), substantial “pre-treatment” data on mule deer productivity, survival, and cause-specific mortality (Pojar, Watkins, Bishop). Historical puma research (Anderson et al. 1992). Substantial number of people live in the eastern foothills and along the eastern, western, and southern edges of the plateau. Domestic sheep (~6 operators), cattle, and horses use the area. Puma hunting pressure is moderate. Puma predation on domestic animals is low.

Puma can be captured year-round using 4 basic methods: trained dogs, cage traps, foot-hold snares, and hands (for small cubs). Capture efforts with dogs will be conducted mainly during the winter when snow facilitates 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, and walking. When puma tracks ≤1 day old are detected, trained dogs will be released to trail puma. 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 3.3 mg/kg estimated body mass (Wildlife Restraint Handbook, 1996, California Dep. of Fish and Game, Wildlife Investigation Laboratory, Sacramento). Immobilizing agent will be delivered in a Pneu-Dart® shot from a CO<sub>2</sub>-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  $\approx$  70—80 bpm, respiration  $\approx$  20 bpm, capillary refill time  $\leq$  2 sec., rectal temperature  $\approx$  101°F average, range = 95—106°F.) (Wildlife Restraint Handbook, 1996, California Dep. of Fish and Game, Wildlife Investigation Laboratory, Sacramento).

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 (Sweaner et al. 2004). Efficiency of the trap will be enhanced by using an automated digital call box that emits puma vocalizations (Wildlife Technologies, Windham, NH). Researchers will 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 with a pole syringe. Immobilized puma will be restrained and monitored as described above.

Foot-hold snares will be used to capture adults, subadults, and large cubs as described by Logan et al. (1999). Puma will be immobilized with Telazol injected with a pole syringe and their vital signs monitored during the handling procedures. Efficiency of snares will also be enhanced with the use of an automated digital call box.

Small cubs ( $\leq$ 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  $\approx$ 100 m to minimize disturbance and human scent at nurseries. Immediately after handling processes are complete, cubs will be returned to nurseries (Logan and Sweaner 2001).

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 Sweaner, unpubl. data). Ages of subadult and cub puma will be estimated initially based on dental and physical characteristics of known-age puma (Logan and Sweaner 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-tag), blood (30 ml from the saphenous or cephalic veins), and hair (from various body regions) for genotyping individuals, parentage analysis and disease screening; fecal for diet analyses. Universal Transverse Mercator Grid Coordinates on each captured puma will be fixed via Global Positioning System (GPS, North American Datum 27).

#### Marking, Global Positioning System and Radio-telemetry- Objectives 1—9

Puma do not possess easily identifiable natural marking, such as tigers (see Karanth and Nichols 1998, 2002), therefore, the capture and marking of individual puma is essential to a number of program objectives. Adult and subadult puma will be marked 3 ways: radio-collar, ear-tag, and tattoo. The identification number tattooed in one pinna is permanent and cannot be lost unless the pinna is severed. A colored, numbered 25 mm diameter ear-tag will be inserted into the other pinna to facilitate individual identification during recaptures and in photos taken by field cameras (see capture-recapture methods below).

Adult and subadult puma will be fitted with GPS collars (approximately 400 g each, Lotek Wireless, Canada) programmed to fix and store puma locations at least 4 times per day at 6-hours intervals to sample daytime, nighttime, and crepuscular locations. Each collar will have a color-coded identification number on each side also to facilitate identification during physical recaptures and photographic resightings. GPS locations for puma will provide precise, quantitative data for estimating

puma home ranges, habitat use, quantifying road crossings (an index to vulnerability to hunting), finding ungulate kills (at location clusters), and estimating kill rates on ungulate prey (i.e., days per kill). 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 will have a mortality mode set to alert researchers when puma have been immobile for at least 4 hours so that dead puma can be found to quantify survival rates and agent-specific mortality rates by gender and age.

At least one cub of each sex in each litter will be fitted with small VHF transmitter mounted on an expandable collar ( $\approx 100\text{g}$ , MOD 210, Telonics, Inc., Mesa, Arizona). Simultaneous locations of mothers and radioed cubs enable researchers to quantify the frequency that mothers are away from cubs to assess the potential risk of orphaning by hunters and other mortality factors, and quantification of survival rates and agent-specific mortality rates. Attrition of cubs in the remainder of the litter can be estimated by periodic visual checks for other siblings by homing on radioed cubs (Logan and Sweanor 2001).

Locations of GPS- and VHF-collared puma will be fixed at least 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 and UTM's along with location attributes will be recorded on standard forms. GPS locations will be mapped using ArcGIS 8 software.

#### Puma Abundance— Objectives 1, 4 & 5

##### *Capture-recapture estimates*

1. *Capture-recapture models* will be used to estimate the parameters of primary interest— absolute numbers of independent puma (i.e., number of puma present in the survey area) and puma density (i.e., number of puma/100 km<sup>2</sup>) each winter— Dec. through Mar.— when snow facilitates detection and capture of puma, provided that we meet model assumptions. The Dec.—Mar. 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 of legal harvest age. Furthermore, adults comprise the breeding segment of the population and subadults comprise potential recruits into the adult population in  $\leq 1$  year. Thus, the sampling unit is the individual independent puma ( $\approx \geq 1$  yr. old).

General assumptions for 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).

Marked puma will make it possible to acquire most of the basic statistics needed for capture-recapture models. Those include:  $n_j$  (number of individually identified puma caught and released on occasion  $j$ ),  $m_j$  (number of previously marked puma recaptured in occasion  $j$ ),  $u_j$  (number of new unmarked puma captured in occasion  $j$ ) (Otis et al. 1978, White et al. 1982). Attribute data of captured puma, such as age and sex, will be recorded to stratify the population in case separate analysis of different strata is necessary (if sample size allows) to meet certain assumptions of capture-recapture models (Pollock et al. 1990). Precise estimates of puma population size may also allow analyses of functional relationships between population vital rates and population size.



We anticipate it may take 2 years to capture and mark the large majority of puma in the population. Our operational objective will be to have  $\geq 90\%$  of the independent puma marked before capture-recapture occasions commence. Capturing and marking puma is time consuming, and would lengthen the time to thoroughly search the study area for capturing and marking puma during the capture-recapture occasions, therefore, we will capture and mark puma prior to performing capture-recapture occasions. 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 during the winter. 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). Our year-round capture efforts using trained dogs, foot-hold snares, and cage traps should help to reduce biases in capture probabilities attributed to any individual capture method (Miller et al. 1997). Thus, capture-recapture occasions may not begin until the end of the second winter. Capture-recapture 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, biases in field methods (relative to GPS data on collared puma), and precision of capture-recapture models used to estimate the puma population.

Data gathered directly from GPS-collared puma and knowledge of the study area acquired by the research team in years 1—2 will allow us to assess if capture-recapture methods are appropriate (i.e., if basic assumptions can be met), and if they are, facilitate the exact design of the mark-recapture schemes for population and density estimates. Movements of GPS-collared puma in and out of the study area during capture occasions will also allow us to estimate corrections for such movements (White 1996).

A composite range (i.e., minimum convex polygon) of all the GPS-collared puma home ranges (i.e., using locations from each of the collared puma) will be estimated and mapped to define the search area (i.e., the area inhabited by the estimated population) and allow mapping of search routes for a thorough systematic search of the area to detect puma for capture (i.e., any individual puma should not have a negligible probability of detection). Density estimates using the generated population estimates will be based on the search area (i.e.,  $N/\text{Area}$ ) (Miller et al. 1997).

A grid will be constructed on the same search area with cells equal to the minimum home range size. There will be a minimum of 4 search routes per cell, each chosen to sample a quarter of each cell. Any spaces in habitat on the search area not occupied by collared puma will also be sampled.

Capture occasions will be repeated 3—6 times each winter (i.e.,  $t_1, t_2, t_3, \dots, t_6$ ) to resample the population. Unmarked puma will be marked and returned to the population to increase the precision of population estimates. Teams of trained houndsmen (4—6 teams of at least 2 persons each) will be used to systematically and thoroughly search the study area each occasion. Capture occasions will be about 1—2 weeks apart. Capture occasions will commence 1—2 days following fresh snowfall that covers the study area and last 3—5 days (i.e., this is how long it may take teams to thoroughly search the study area). But if fresh snowfall is lacking, we will attempt capture efforts anyway (although this could increase variation in capture probabilities).

At puma captures, the puma identification number, sex, age, and location (U.T.M. coordinate) will be the minimum information recorded. If the same individual puma is caught more than once in the same occasion, each capture will be recorded, but only the first capture will be used for data analysis. All capture-recapture occasions will be conducted within a 2—3 month span in winter to minimize the chance of population changes (i.e., deaths, immigration, emigration). Once capture history data on puma are gathered, estimates of the number of independent puma in the population in winter can be made by using capture-recapture models that deal with variation in capture probability and closed or open populations (Otis et al. 1978, White

et al. 1982, Pollock et al. 1990). In order for closed models to be valid, the population of independent puma cannot change (as a result of death, emigration, or immigration) during the 2—3 month span that contain the capture-recapture occasions.

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 about 0.6 (for 3 occasions) and 0.4 (for 6 occasions) to yield capture probabilities  $\geq 0.9$  for individual puma in the population each winter (Trolle and Kery 2003). Theoretically, capture probabilities within this range (i.e., 0.4—0.6) would tend to reduce the coefficient of variation of the estimate to about 0.20 (i.e., increase the precision of the estimate) in small populations where individuals have a survival probability of about 0.90 in 5 samples (Pollock et al. 1990:72), which is realistic for puma.

In addition, behavior, movements, and survival rates of GPS-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 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 may be used (Pollock et al. 1990). 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 as well. This will allow us to determine the number of marked puma present in the search area each capture-recapture occasion and the probability that unmarked puma move in and out of the search area during each occasion.

2. *Photographic captures and recaptures* (i.e., camera traps) could be assessed in a 3-year pilot study (e.g., years 2—4) as an independent method for estimating puma numbers and density on the study area (Mace et al. 1994, Karanth 1995, Karanth and Nichols 1998, Karanth and Nichols 2001, Trolle and Kery 2003). The pilot study could be carried out by a graduate student and begin in the second winter, at which time we may begin estimating the puma population using capture-recapture methods (described above).
3. *Genotype captures and recaptures* could be assessed in coordination with the physical and photographic capture methods described above (1 and 2). This could also be initiated as a 3-year pilot study (e.g., years 2—4) done by a graduate student. Genotypes can be used to estimate minimum population size directly (Kohn et al. 1999) and in capture-recapture models (e.g., Otis et al. 1978, Boulanger et al. 2002). However, genotyping errors should be estimated and considered in population estimates (Creel et al. 2003). This project could be another independent non-invasive method for estimating puma numbers and density on the study area. Moreover, this method may be useful to monitor puma populations in Colorado (see Indices to Puma Abundance below).

### Vulnerability of Puma to Hunters— Objective 3

Puma hunting in Colorado normally involves hunters searching for puma tracks while driving snow-covered roads with four-wheel drive vehicles or snowmobiles. Thus, vulnerability of puma to hunters is associated with the frequency that puma cross roads (Murphy 1983, Van Dyke et al. 1986, Barnhurst 1986). Hunters active on snow may successfully catch puma >85% of the time that they release dogs on tracks, and road access influences puma hunting distribution (Murphy 1983).

Road crossing frequencies of GPS-collared puma will be used to assess relative vulnerability of various sex, age, and reproductive classes of puma to detection by puma hunters. Density of roads (i.e., km/100 km<sup>2</sup>) will be estimated within each GPS-collared puma home range (i.e., 100% minimum convex polygon), each quadrat in the sampling frame, and the entire study area. Road crossings per puma per 24-hour periods will be quantified (GPS collars programmed for 4 locations per day, each 6 hours apart). Comparisons will be made between road crossing frequencies of puma classes and road densities in home ranges for puma classes. Puma classes will be: adult females ( $\geq 2$  yr. old) with no cubs; adult females with cubs  $\leq 2$  months old (i.e., nursing cubs), adult females with cubs 3—12 months old (i.e., weaned, carnivorous cubs), subadult females (i.e., independent females  $< 2$  yr. old), adult males (i.e.,  $\geq 2$  years old), and subadult males (i.e., independent males  $< 2$  years old) (Logan and Sweanor 2001).

Vulnerability will also be quantified using road crossing frequencies of different classes of puma (female, male, divided into adult and subadult age classes if individuals are known) during the track index discussed below. In addition, actual capture rates of those puma will be quantified during capture-recapture occasions (no. of captures per puma class/no. of puma in each class). Frequency of capturing known puma mothers with and without their cubs will also be tallied to quantify vulnerability of puma mothers to legal harvest (i.e., known mothers without cubs by their side).

During the puma population decline (i.e., reduction) phase (years 6—10), hunters can be used to kill puma. Relative vulnerability to and selection by hunters will be quantified during hunting seasons by having hunters report number of hunter-days, number of tracks encountered and locations (fixed by GPS), number of times dogs were released on tracks, number of captures, characteristics of killed or captured puma (i.e., hunters may capture puma and release them). At the same time, researchers will have quantitative knowledge of puma available for harvest on the study area (as a result of ongoing capture and marking procedures and GPS data) to estimate capture rate per puma class. The hunter-kill will also allow a direct assessment of puma mothers in the harvest and a comparison of the fates of potential orphaned cubs with cubs in intact families.

#### Indices to Puma Abundance— Objective 5

This project will develop and test both the efficacy and feasibility, including costs and other management considerations, of using indices to monitor changes in puma abundance. Two such indices are track counts and catch-per-unit-effort. These indices will be calibrated with the estimated puma population.

##### *Track Counts*

An index to puma abundance using counts and classification of puma tracks on snow-covered routes will be developed and tested. This will be done simultaneous with obtaining estimates of puma population size using capture-recapture occasions with houndsmen teams (above) and spatial analysis of home ranges of GPS-collared puma.

A 3-year pilot study for this index may be started in year 2 and could be conducted by a graduate student. Information on puma gathered during years 1—2 will facilitate the exact research design. Experimental manipulation of the puma population resulting in a 5-year increase phase and a 5-year decline phase will allow testing the index through known puma population changes and assessment of the sensitivity of the index with the parameter in question— puma population size— by analyzing the statistical power of the method to detect population change (up or down) (Kendall et al. 1992, Beier and Cunningham 1996).

The main operating assumption is that the frequency of finding puma tracks on snow-covered routes is related to puma numbers. We will examine the number of individual puma track sets/km (differentiated by size and direction of travel) and presence of puma tracks/km of search route to see

which metric better detects actual population change (Kendall et al. 1992). In addition, we will examine how frequency of different classes of tracks (male, female, females with cubs) may relate to the known puma population changes. The most direct relationship will be a linear one between puma numbers and frequency of encountering puma tracks. Snow-tracking conditions (e.g., powder, crusted, slush, continuous, patchy) will be categorized each search day.

Tracking teams, different than houndsmen teams used in capture-recapture occasions, will be used to make the puma track surveys 3 to 6 times per winter. Track surveys will be run on 4-wheel drive vehicles or snowmobiles 1—2 days following snowfall that covers the study area. Effort and costs will be quantified.

#### *Catch-Per-Unit-Effort*

The main operating assumption is that the amount of effort to capture puma is related to puma numbers (Lancia et al. 1996). The puma research team(s) will quantify the number of days required to capture individually identified puma with dogs during each year in the population increase phase. We will also quantify the number of days required to capture unmarked puma with dogs each year, and treat marked puma as though they have been removed from the unmarked population. Number of days per capture will also be quantified by capture teams involved in the capture-recapture occasions each winter.

During the decline phase, puma hunters used to reduce the puma population will quantify the number of hunt days per puma captured each winter. Theoretically, the number of days per puma capture should increase as the puma population is reduced by 20% increments in years 6 and 7, and in possible further reductions in years 8—10. Hunters will also be asked to record (i.e., GPS location, date) the total number of tracks of independently-traveling puma (classified as male and female), and the number of tracks of female puma and cubs they encounter during their hunting periods. Male and female track categories will be distinguished by the width of the hind foot plantar pads. Hind foot plantar pad widths that are >52 mm will be classed as male; hind foot plantar pad widths ≤52 mm will be classes as female. We will explore functional relationships of these efforts to the estimated puma population on the study area.

#### Quantifying Puma Diet and Ungulate Kill Rates— Objective 8

Data collected on puma diet and ungulate kills are not directly pertinent to a puma population study. However, they would be basic to an integrated study that involves effects of puma predation on mule deer and elk. Location clusters where puma are located for ≥2 nights will be investigated to estimate puma kill rates of ungulate prey (i.e., days/ungulate kill type/puma class) (Anderson and Lindzey 2003). Sex of animals will be determined by secondary sex characteristics and ages will be estimated from tooth eruption patterns (Quimby and Gaab 1952, Robinette et al. 1957, Dimmick and Pelton 1996) and cementum annuli of incisors (Low and Cowan 1963).

Necropsies will be performed on all ungulate prey recovered in the field (Roffe et al. 1996), whether killed by puma or not, and data will be recorded on standard forms. If disease is suspected, whole carcasses or vital organ tissues will be collected and preserved by standard procedures (Roffe et al. 1996) and submitted for analysis to the Colorado Division of Wildlife's Wildlife Health Laboratory or the Colorado State University Diagnostic Laboratory. An index to physical condition of ungulates prior to death will be estimated from percent marrow fat in femurs or metatarsi (depending on presence; femurs are preferred) (Neiland 1970, Mech and DelGiudice 1985, Fuller et al. 1986, Husseman et. al. 2003).

Puma feces will be collected opportunistically year-round and stored by either freezing or oven drying (80-85°C, then stored in paper bags with a fumigant) for later macroscopic diet analysis (Big Sky Laboratory, Florence MT) to estimate frequency of occurrence of prey species (Litvaitis et al. 1996). This research component could also be carried out by a graduate student.

### Behavior of Puma Subject to Aversive Conditioning— Objective 9

Information on responses of puma to aversive conditioning is lacking. Individual puma with activities in residential areas on the study area might be research subjects on effectiveness of aversive conditioning. GPS collars on puma would be the primary source of behavioral response data before, during, and after aversive conditioning treatments.

### Behavior and Survival of Translocated Puma— Objective 10

If translocation is chosen as the method of reducing the puma population during the decline phase (years 6—10), then researchers will remove puma at rates needed to test research hypotheses. Prior to translocation, potential puma habitat areas for the release of the puma will need to be identified which are: 1) relatively remote, 2) large enough to accommodate exploratory movements up to 84 km away from release areas, and 3) not near human residential areas, domestic animal operations, or desert bighorn sheep populations (Ruth et al. 1998, Logan and Sweanor 2001). Puma will be captured alive on the study area, fit with new GPS collars, transported in wooden crates, provided food and water, and translocated by truck a minimum of 120 airline km (75 mi.) for females and 220 airline km (137 mi.) for males (Ruth et al. 1998). GPS collar locations will allow researchers to map movements of translocated puma. The VHF transmitters will allow researchers to quantify survival rates and agent-specific mortality rates. This research could also be carried out by a graduate student.

## **ANALYTICAL**

Puma class survival rates and agent-specific mortality rates will be estimated by using Kaplan-Meier (Pollock et al. 1989a, b) and Trent and Rongstad procedures (Micromort software, Heisey and Fuller 1985). Cub survival curves for each gender will also be plotted with survival rate on age in months (Logan and Sweanor 2001:119).

To analyze capture-recapture, photographic, and genetic capture-recapture data, closed population capture-recapture models are available in program CAPTURE obtainable at [www.mbr-pwrc.usgs.gov/software.html](http://www.mbr-pwrc.usgs.gov/software.html) and program MARK obtainable at [www.cnr.colostate.edu/~gwhite](http://www.cnr.colostate.edu/~gwhite). Closed population model selection can be achieved with the algorithm based on goodness-of-fit tests and between model tests in program CAPTURE (Otis et al. 1978). For open populations, programs JOLLY (for 1 age class), and JOLLYAGE (handles 2 age classes) are available at [www.mbr-pwrc.usgs.gov/software.html](http://www.mbr-pwrc.usgs.gov/software.html). Programs JOLLY and JOLLYAGE contain chi-square goodness-of-fit tests of model assumptions and between model tests that enable researchers to choose the most appropriate model for the data (Pollock et al. 1990). NOREMARK (White 1996), also available at [www.cnr.colostate.edu/~gwhite](http://www.cnr.colostate.edu/~gwhite), has an extension that accommodates immigration and emigration; thus, it does not assume geographic closure (but demographic closure is still assumed).

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

Graphical methods will be used to examine relationships of track counts and catch-per-unit effort (i.e., indices to puma abundance) to changes in the population of independent puma. Linear regression procedures and coefficients of determination will be used to assess functional relationships of track counts and catch-per-unit effort to changes in the population of independent puma 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). We will also consider non-parametric correlation methods, such as Spearman's rank correlation coefficient, to test for a monotonic relationship between the index of abundance and the change in the puma population (Conover 1999).

Statistical analyses will be performed using SYSTAT 10.2 and SAS 6.11. The risk of committing a type I error (i.e., concluding that a population change occurred when it did not) will be controlled at  $\alpha = 0.10$  because we will normally have small sample or population sizes (typical of large-carnivore studies). 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.

ArcView 8 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 frame.

### **PRELIMINARY SCHEDULE**

Years 1—5 (2005—2009) will be the puma population increase phase. Protecting the puma population from sport-hunting will be vital to allowing the puma population to increase within the bounds of the ecological carrying capacity of the study area. This will allow researchers to quantify baseline demographic data on the puma population and test indices to puma abundance during an increase phase. In this phase, capture-recapture occasions, track counts (for index to abundance), and photographic and genetic capture-recapture efforts will begin in about year 2 (2006).

Years 6—10 (2010—2014) will be the puma population decline phase. Puma hunters (or translocation) will be used to experimentally reduce the puma population. The portion of independent puma (i.e., adults and subadults) in the population will be reduced by 20% in year 6 and 20% more in year 7 (i.e., a 40% reduction from year 5). Additional reductions may be made to test the indices to abundance or other hypotheses that may be developed and related to effects of harvest or puma predation on mule deer and elk. Those decisions can be made later in project development and as late as years 8—10.

### **REGULATORY NEEDS**

Puma on the study area that may be involved in depredation of livestock or human safety incidences will not be treated any differently than other puma in Colorado, whether they are marked or not. Thus, they 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).

The increase phase in years 1—5 will require a temporary interruption of puma sport-hunting on the study area and protection of radio-collared puma that range off the study area. In years 6—10, regulated puma sport-hunting will resume.

### **POTENTIAL COOPERATORS**

The Colorado Division of Wildlife will be the principal research and regulating agency in this program. The Bureau of Land Management and the Forest Service will be cooperators because the majority of the study area may be on lands under their management jurisdiction. U. S. D. A., A. P. H. I. S. Wildlife Services may provide puma capture assistance. Private landowners on the study area will be asked to cooperate with this effort. Colorado State University and other universities may cooperate by providing graduate research assistants and professors to carry out specific projects of the research program. Private individuals interested in the puma research may be asked to cooperate in puma capture and monitoring operations.

Table 2. Preliminary puma research schedule.

Phase	Increase (Years 1—5)	Decline (Years 6—10)
<i>Objectives:</i>	<i>Methods &amp; Data:</i>	
1.	Initial capture & mark efforts of $\geq 90\%$ of independent puma (yrs. 1—2). Capture-recapture estimates (yrs. 2—5; 3 yr. minimum required for trend) using physical, photographic & genotype captures. Quantify puma population sex & age structure, density, & population growth rate.	Capture-recapture estimates (yrs. 6—10) using data from physical and photographic & genotype captures (if reliable). Reduce puma population using hunting or translocation. Reduce by 20% increments in years 6 & 7. Puma hunting will continue, and there may be additional population reductions in subsequent years. Quantify structure of hunter-kill.
2.	Quantify puma population vital rates as population increases.	Quantify puma population vital rates as population declines.
3.	Quantify agent-specific mortality & puma road-crossing frequency.	Quantify agent-specific mortality & vulnerability and selectivity of puma to hunters.
4.	Develop and test puma population models validated by observed increase phase puma population.	Develop and test puma population simulation models validated by observed decrease phase puma population.
5.	Use track counts, catch-per-unit effort, & genotype capture-recapture methods as indices to puma abundance (yrs. 2—5).	Use track counts, catch-per-unit effort, & genotype capture-recapture methods (if reliable) as indices to puma abundance.
6.	Use GPS data to quantify puma activity in relation to people and human facilities on the study area.	Use GPS data to quantify puma activity in relation to people and human facilities on the study area.
7.	Use GPS data to quantify puma use of habitats and landscape linkages.	Use GPS data to quantify puma use of habitats and landscape linkages.
8.	Estimate puma kill rates on mule deer and elk using GPS data. Quantify puma diet from feces.	Estimate puma kill rates on mule deer and elk using GPS data. Quantify puma diet from feces.
9.		Describe & quantify behavior & survival of translocated puma if translocation is used to reduce the puma population. Begin final data analysis & report year 10.

### POTENTIAL IMPEDIMENTS

Because of the relatively low densities of puma, difficulty of capture and research, obtaining needed sample sizes is expensive. Furthermore, multiple years of study are requisite to fulfill objectives. Collared puma that are killed, therefore, represent a significant effort and data loss. Minimizing such losses is a challenge that will improve the efficiency of the study. For certain projects within the program, experimental manipulations of the puma population on the primary study area, possibly ranging from extreme protection to extreme suppression at different stages of the project, are necessary to maximize reliability and scientific defensibility of findings.

## FINANCIAL ESTIMATES

Conducting intensive puma research requires significant and steady financial support. Yearly costs during years 1-5 are estimated to range between \$177,000 and \$355,000 (Table 3).

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Table 3. Estimated project costs for years 1—5 only.

Budget Item	Year 1	Year 2	Year 3	Year 4	Year 5
<i>Personnel:</i>					
-DOW Researcher	K. Logan's support not incl.				
-Houndsman	12,500 (\$2,500/mo.*5 mo.)	12,500	13,125	13,125	13,781
-Project Technician	35,000	35,000	35,000	35,000	35,000
-Temporary Technician	33,000	33,000	33,000	33,000	33,000
<i>Volunteers Support:</i>					
Lodging, food, fuel for 8—12	0	15,000	15,000	15,000	15,000
<i>Vehicles:</i>					
-4x4 Trucks (2)	50,000 (2*\$25,000)	0	0	60,000	0
-all terrain vehicles (3)	18,600 (3*\$6,200)	0	0	0	0
-snowmobiles (1) <sup>a</sup>	5,300	0	0	0	0
-utility trailers (1) <sup>a</sup>	1,900	0	0	0	0
-dog sled & trailer	1,000	0	0	0	0
Gasoline	4,800 (2*\$2,400)	4,800	4,800	4,800	4,800
Vehicle Maintenance	3,000	3,000	3,000	3,000	3,000
<i>GPS- &amp; Radio-telemetry:</i>					
-GPS-collars	114,750 (25*\$4,590)	0	22,950	22,950	22,950
-VHF-collars (cub)	3,192 (12*\$266)	0	1,596	0	1,596
-cub collar material	100	0	50	0	50
-command unit (1)	4,500	0	0	0	0
-receivers, type (2)	5,390 (2*\$2,695)	0	0	0	0
-H-antennae (3)	636 (3*\$212)	0	0	0	0
-omni antennae (3)	234 (3*\$78)	0	0	78	0
-coaxial cables (2)	56 (2*\$28)	0	0	56	0
-coaxial cables (2)	29 (2*\$14.50)	0	29	0	29
-antenna switch-box (2)	112 (2*\$56)	0	0	56	0
-intercom system (1)	285	0	0	0	0
-head sets (2)	300 (2*150)	0	0	0	0
<i>Capture Equipment:</i>					
-drugs	650 (25*\$26/bottle Telazol)	650	650	650	650
-cage trap	2,000 (2*\$1,000)	0	0	0	0
-snares	1,000	0	0	0	0
-call box	850	0	0	0	0
-miscellaneous (darts, vials, syringes, needles, envelopes, gloves, tapes, calipers, thermometers, ear-tags, tattoos, etc...)	1,000	1,000	1,000	1,000	1,000
<i>Dog Care:</i>					
-veterinary	2,000	2,000	2,000	2,000	2,000
-food	600 (120/mo.*5 mo.)	600	600	600	600
Aerial Support:	40,000 (\$200/hr x 4 hr x 50)	40,000	40,000	40,000	40,000
<i>Work tack:</i>					
-backpacks, climbing gear, nets, ropes, office materials, etc...	2,000	1,000	1,000	1,000	1,000
<i>Laboratory:</i>					
-genetics	4,650 (25*\$186)	4,650	4,650	4,650	4,650
-carcass or tissue analysis	600	600	600	4,650	600
-fecal analysis	4,650	4,650	4,650		4,650
<i>Photographic:</i>					
-trail cameras (~40)	0 (40*\$430)	17,200	4,300	0	0
-film (~200)	0 (250*\$6)	1,500	1,500	1,500	1,500
Total	354,684	177,150	189,500	243,715	185,856

<sup>a</sup> Two snowmobiles and 1 trailer are already available for the project.

## APPENDIX I

### Sex Determination of Mountain Lions Bayed in Trees

With little effort the sex can be determined for a mountain lion bayed in a tree. Refer to the photos of the different lions, 4 males (A—D) 2 females (E, F), attached to these tips.

Male adult and subadult lions have a conspicuous black spot of hair, about 1 inch diameter, surrounding the opening to the penis sheath behind the hind legs and about 4 to 5 inches below the anus. In between the black spot and the anus is the scrotum, which is usually covered with silver, light brown, and white hair. Therefore, look for the black spot and scrotum. The anus is usually hidden below the base of the tail.

Female adult and subadult lions *do not* have the black spot or scrotum behind the hind legs and below the base of the tail. There is just white hair there. The anus is directly below the base of the tail, and the vulva is directly below the anus. The anus and vulva are usually hidden by the base of the tail. Teats of females are usually inconspicuous, even of mothers with weaned cubs or mothers that have just finished nursing cubs. So teats are usually not a good indicator of sex in treed lions.

Sometimes sex determination of lions can be done with the naked eye. But use a pair of binoculars to make sexing lions easier. If a lion's position in a tree obscures your view, you can get the lion to move around for a better look. Pick up a baseball-bat-size branch and bang on the trunk of the tree. If there is snow on the ground, throw a few snow balls at the lion. You can even climb the tree toward the lion. These actions usually get the lion to move. When it does, be ready to sex the lion.

Also, sometimes the lion urinates when bayed by dogs or when a person climbs the tree toward it. Look for the origin of the urine stream. If the urine stream comes from behind the hind legs about 4 to 5 inches below the anus, then the lion is probably a male. If the urine stream comes from under the base of the tail, then it's probably a female.

Tracks may also be indicative of sex. Adult and large subadult male lions usually have hind foot plantar ("heel") pad widths that exceed 2 1/16 inches (52 mm). Adult and subadult female lions usually have hind foot plantar pad widths less than or equal to 2 1/16 inches. Carry a small ruler or wind-up metal tape in your pocket to make measurements

**Male Mountain Lions (A—D)**

Penis Spot, Scrotum, Anus. Penis (black) spot ~1 inch dia. is ~4-5 inches below anus.



**A**

K. Logan photo



**B**

K. Logan photo



**C**

K. Logan photo



**D**

K. Logan photo

**Female Mountain Lions (E, F)**

Vulva directly below anus, both usually hidden by base of tail. No “black spot” 4-5 inches below anus



**E** K. Logan photo



**F**

K. Logan photo



Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. \_\_\_\_\_ : Mammals Research  
Work Package No. 3004 : Other Ungulate Conservation  
Task 2 : Potential Research Project Assessment

Federal Aid Project: N/A :

Period Covered: July 1, 2003 through June 30, 2004

Author: Eric J. Bergman and Gary C. Miller

Personnel: D. Freddy, C. Bishop, B. Watkins, J. Madison, J. Broderick

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### ABSTRACT

As part of the research planning process, general assessments were made for the potential to conduct research projects on 3 main topics identified by Colorado Division of Wildlife field management personnel. These topics were: impacts of mule deer/elk interactions on mule deer population performance; improving success of bighorn sheep reintroductions and translocations; and, impacts of natural gas and oil extraction on mule deer. All 3 topics present challenges to conducting successful research endeavors with deer-elk interaction studies potentially providing the most predictable research and funding situations.

## **JOB PROGRESS REPORT POTENTIAL RESEARCH PROJECT ASSESSMENT**

Eric J. Bergman

### **INTRODUCTION**

The Colorado Division of Wildlife is charged with protecting, preserving and enhancing Colorado's ungulate populations for the use, benefit, and enjoyment of the people. The management principles guiding managers in this mission include: wildlife conservation, use and enjoyment, maintaining healthy, diverse and abundant populations and maintaining/conserving habitat quality/quantity through science-based decision making (Colorado Div. of Wildlife 2002-2007 Strategic Plan:9). The objective of ungulate research is to provide information to facilitate the making of these science-based decisions.

### **METHODS**

Members of the Division's Mammals Research staff met with wildlife managers and biologists from the Northwest and Southwest Regions to consult on ungulate management issues and research needs. The following topics were identified as the primary statewide issues of concern:

- Negative impacts of deer/elk interactions on deer population performance.
- Variable success of bighorn sheep reintroduction/translocation efforts.
- Impacts of natural gas and oil extraction on deer.

In general, research topics identified by wildlife managers favored issues occurring in their respective areas and regions. Topics identified by biologists emphasized need for broad-scale research efforts to address issues that exist throughout the state. The objective of research project selection is to successfully merge these two viewpoints into a study that:

- 1) Provides clear results through control/treatment experiments.
- 2) Addresses issues of local and statewide concern.
- 3) Allows inference to wide spatial/temporal boundaries.

### **RESULTS AND DISCUSSION**

#### **Impacts of Deer/Elk Interactions on Deer Population Performance**

A general long-term trend of mule deer population declines has existed both in Colorado and throughout the Western U.S. since the late 1950s (Unsworth et al. 1999, Gill 2001). While insular deer herds have functioned outside of this trend and others have experienced pulses of population growth nested within this long-term decline, declines of as much as 50% have been reported (Colorado Division of Wildlife, unpublished data). Along with these overall population declines, depressed fawn:doe ratios have been simultaneously measured (Gill 2001, White et al. 2001). In an attempt to address concerns over these declines and to develop working hypotheses as to the underlying causes, CDOW hosted a conference for employees of CDOW, CSU, federal agencies, invited publics and deer experts in 1998. A result of this conference was the identification of five potential sources of mule deer population depression: A) habitat deterioration, B) predation, C) competition with elk, D) disease, and E) weather (Fig. 1).

Based on a coupling of the ideas from this conference with existing knowledge of mule deer/coyote predation dynamics (Bartmann et al. 1992), CDOW entered a collaborative mule deer



research program with Idaho Fish and Game. Two studies were simultaneously developed. Idaho began a mule deer predation study that is currently assessing the impacts of predator control (both puma and coyote) on mule deer survival. The Division of Wildlife began a nutrition study to simulate and quantify the demographic impacts of habitat enhancement on mule deer fawn survival (Bishop 2003). Through a treatment/control, cross-over study design, CDOW is providing supplemental forage as a mechanism to test the effects of an immediate, short-term enhancement of mule deer habitat (Bishop 2003). Preliminary results of this ongoing research indicate that by enhancing the nutritional value of mule deer diets, overwinter fawn survival can be increased (Bishop 2003). While the methods employed through this research are not an acceptable management strategy to the Division, the pending conclusions exemplify the need for landscape level treatments to enhance mule deer habitat (discussed below).

As such, through a series of reductionist experiments, it has been demonstrated that mule deer population performance is directly related to habitat quality, yet functions somewhat independently of coyote predation. However, a confounding factor in these conclusions stems from the fact that concurrent with mule deer declines in Colorado, there has been a dramatic increase in the distribution, number and density of elk. Accordingly, while enhancing the quality of habitat should improve mule deer survival, the ultimate causes of habitat degradation have not been tested. It remains unclear whether interference/exploitation by elk has further reduced carrying capacity for mule deer in late seral stage pinyon-juniper ecosystems.

Based on past research, there is variable evidence that competition between mule deer and elk exists. Both interference and exploitation competition have been reported for deer and elk (Singer and Norland 1994, Kirchoff and Larsen 1998, Stewart et al. 2003). However, due to logistical constraints, these studies have been observational and are highly dependent on the specific conditions at which the data was collected. Thus, strong inference is not possible. In order to accurately quantify the impacts of deer/elk competition, a study would need tight control over ungulate densities. Under ideal conditions, this would be done in a controlled setting (i.e. large enclosures). Due to financial constraints and disease potential, this is not possible in Colorado. Additionally, enclosures with controlled densities of deer and elk remove much of the natural variation that exists in nature. Elk are highly mobile animals with weak site fidelity. Thus, while deer may encounter high elk densities one week, they could very easily encounter no elk the following week. Enclosures with static deer and elk densities would remove this variability and subsequent results would be of limited utility. Elk mobility is also the primary reason that density reduction treatments cannot be applied in field settings. A dramatic harvest treatment to reduce local elk density could be erased as elk immigrate into the treatment area once harvest is finished.

Given these constraints, the possibility of studying the interactions between deer and elk still exists. Currently, the potential to do this is presenting itself on the Uncompahgre Plateau. The proposed follow-up to the short-term deer nutrition research is the implementation of large scale deer habitat enhancement treatments. However, there is concern that elk will monopolize treatment areas and will either displace deer or deprive deer of receiving the intended treatment. As such, regardless if elk are being studied, mitigation efforts will have to be employed to insure the delivery of treatments to deer. Due to the baseline knowledge on deer in the Uncompahgre system (C. Bishop, unpublished data, B. Watkins, unpublished data), the existing capital investment and given the fact that elk treatments would be an integral part of any deer habitat enhancement study, many of the financial and logistic hurdles to starting a complimentary elk research program would be minimized.

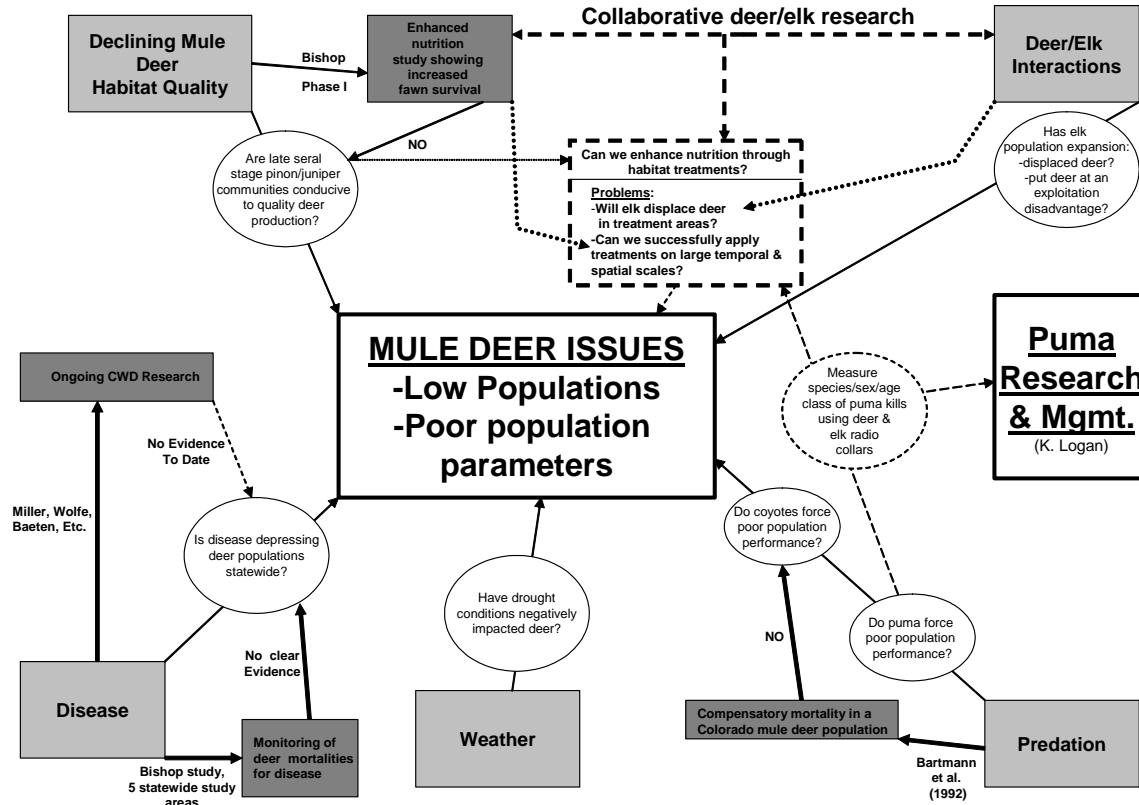


Figure 1. Conceptual model concerning mule deer population declines in Colorado with an emphasis on the relationships between sources of population depression (light gray boxes), current and past research (dark gray boxes) and potential research (dashed lines).

A final benefit to conducting a study on the interactions between elk and mule deer on the Uncompahgre Plateau pertains to ecosystem level understanding. As the likelihood of puma research being conducted on the Uncompahgre Plateau increases, the addition of elk research would allow for integrated, broad scale understanding of deer, elk and puma management. Over the past 25 years, wildlife ecologists have strived to understand ecosystem level interactions (Sinclair and Norton-Griffiths 1979, Houston 1982, Jedrzejewska and Jedrzejewski 1998, Krebs et al. 2001). However, a fundamental feature of these studies is that they were conducted in systems that were managed for species conservation (i.e. no hunting), limiting the utility and applicability in systems where species are managed as a consumptive resource. All elements will likely be in place to do ecosystem level research in a system that is managed with a multi-use strategy.

The level of interest within the CDOW and other western state agencies in studying deer/elk interactions is high (D. Freddy, unpublished study plan, 1998). Additionally, based on high priority achievements H-1.2 and H-1.3 of the Colorado Division of Wildlife Strategic Plan (2002-2007), research on these issues are highlighted as guiding principles of Division activities. This interest is being echoed by external conservation groups. Over the past decade, the Uncompahgre Project of southwest Colorado has actively pursued habitat enhancement on the Uncompahgre Plateau. This group has expressed interest in coordinating their proposed habitat manipulations with wildlife research, potentially relieving the Division of the financial burden of conducting landscape level treatments (R. Sherman, personal communication). Additionally, it is believed that groups such as the Mule Deer Foundation and the Rocky Mountain Elk Foundation would be willing to invest in research that further explains the impacts of deer/elk interactions as well as the effectiveness of habitat enhancement for these species.

### **Variable success of Bighorn Sheep Reintroduction/Translocation Efforts**

During the late 1800's and early 1900's, the rocky-mountain west experienced dramatic declines in bighorn sheep populations (Singer et al. 2000a). Due to the role that bighorns play in ecosystems and their value as both a watched and hunted species, recovery is a high priority. Approximately 55%-58% of the existing populations are a result of reintroduction efforts (Singer et al. 2000b). However, reintroduction efforts have been variable in success (success rates fall between 40% and 58%) (Singer et al. 2000a, Singer et al. 2000b). While these trends and statistics are for the west as a whole, most are indicative of bighorn management efforts within Colorado (Bailey 1990).

There are many parameters that influence the success of bighorn sheep reintroduction efforts. Among these, survival, density, metapopulation characteristics, habitat quality/quantity, disease and predation have all been identified as potential limiting factors (Fig. 2). However, due to the lack of experimental manipulation, the relative importance of any single parameter is largely unknown.

Bighorn population characteristics are poorly understood, yet are likely important for planning and implementing successful recovery efforts. Many recovery efforts appear to be typified by brief periods of very high population growth, followed shortly by stabilization and population crash (Singer et al. 2000c). In the absence of disease, adult survival is typically high. However, lamb survival can be impacted by density as well as weather (McCarty and Miller 1998, Holl et al. 2004). In the absence of dispersal corridors, and due to potential growth rates as high as  $\lambda = 1.30$  (Singer et al. 2000c), many recovering bighorn populations suffer from sedentariness. Because bighorns can quickly reach the local carrying capacity of a patch, and due to high adult survival, the instigation of senescence at approximately 7-9 years of age, and low dispersal, it is possible that recovery efforts fail simply because no new individuals are entering the population. A manipulative, research approach to addressing this question is to emulate dispersal through the selective removal of senescent individuals, thereby reducing density and forage pressure such that lamb survival can increase.

Bighorn sheep are thought to have traditionally functioned as a metapopulation, with a single population being characterized as a discrete group with limited movement of individuals between groups. While limited in overall quantity, the role of immigration and emigration is vital in metapopulation stability. Dispersal typically occurs in the form of animals leaving one population to join another existing population (i.e. into contiguous, occupied habitat) (Singer et al. 2000a). While providing relief for dense populations, dispersal also provides other populations with new genetic stock. Unfortunately, most recolonization efforts have focused on filling insular habitat patches with a single population and dispersal is not considered. By taking a multi-patch view of the landscape and populating several patches with animals, a metapopulation could be established.

Bighorn sheep habitat is typified by open vegetation structures with high visibility and rough terrain to avoid predation (Singer et al. 2000a). Additionally, typical bighorn sheep habitat is composed of climax (late-seral stage) plant communities. These habitats occur naturally (as well as through human influence) in a fragmented fashion across the landscape, further explaining the metapopulation structure. Movement between habitat patches is easily arrested by physical barriers such as rivers and roads (Singer et al. 2000b). Absence of these key habitat characteristics and presence of barriers are all factors that potentially have a negative impact on recovery efforts. Unfortunately, experimental research has not been conducted to determine the actual importance of any single parameter.

Disease has also been identified as a potential arresting factor in recolonizing bighorn populations. Epizootic breakouts can reduce bighorn sheep populations by >20%/year (McCarty and Miller 1998). Typically epizootic breakouts are in the form of *Pasturella haemolytica*, however, parainfluenza-3 and other *Mycoplasma* outbreaks have also been documented (Singer et al. 2000c). In Colorado, lungworm (*Protostrongylus* spp.) has also been prevalent. Diseases such as bacterial

pneumonia are often introduced to bighorn populations via exposure to domestic sheep (Goodson 1982). In a massive reintroduction program throughout the west during the 1990's, a 16km buffer between wild and domestic sheep populations was deemed necessary for habitat to be classified as suitable for bighorn reintroductions (Singer et al. 2000a). Precautionary vaccination programs have been instituted, but reported results have not indicated that such approaches greatly enhance bighorn population recovery (Miller et al. 2000).

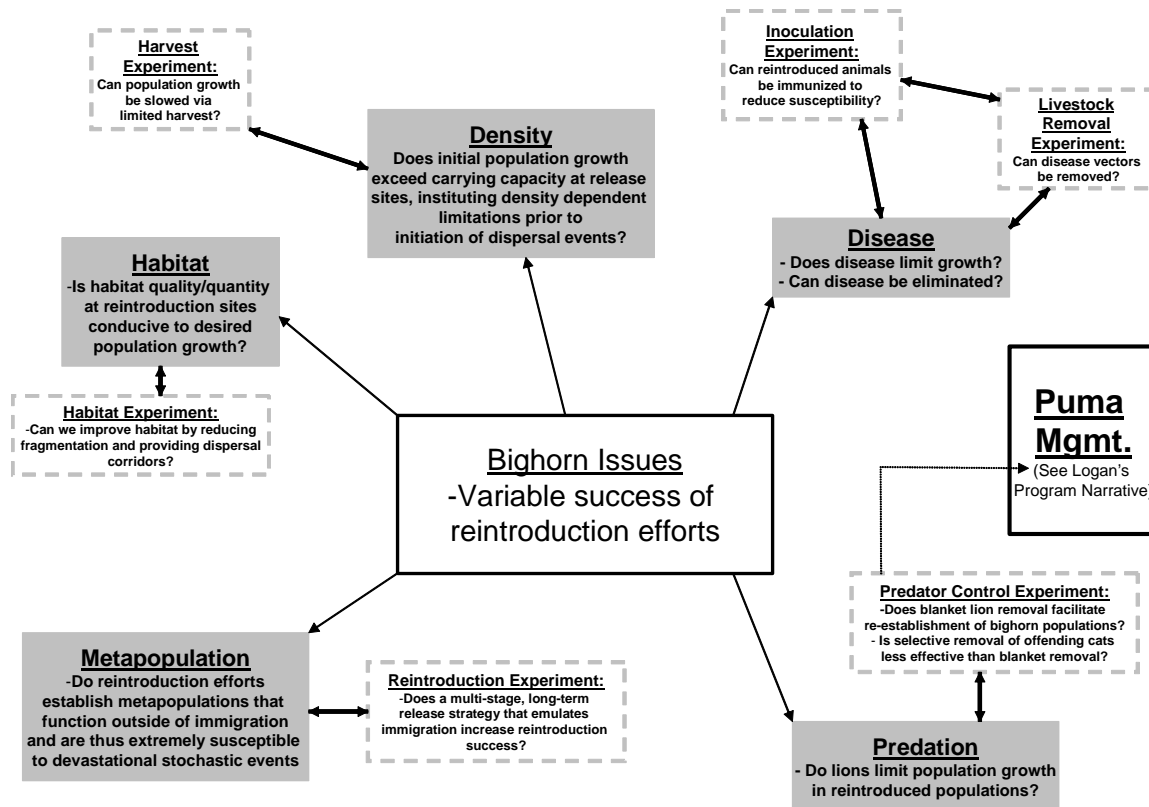


Figure 2. Conceptual model concerning the variable success of bighorn sheep reintroduction efforts with emphasis on the relationships between limiting factors (gray boxes) and research possibilities (dashed lines).

The role of puma predation on bighorn sheep populations is heavily debated in the literature, but in some circumstances is thought to be the limiting factor in bighorn population growth (Wehausen 1996, Hayes et al. 2000, Holl et al. 2004). In Colorado, the impacts of puma predation on desert bighorn sheep are an issue of concern. For instance, it is believed that recent desert bighorn recovery efforts in the Dolores canyon were unsuccessful due to puma predation. Of the 12 radio-collared animals in this population, 11 have died. Nearly 100% of these deaths were due to predation (those not classified as predation were classified as unknown) (B. Watkins, unpublished data). Due to this preliminary evidence, an experimental research project that addresses the impact of puma predation on desert bighorns would likely be beneficial to future desert bighorn recovery efforts.

In summary, there are many management-driven experiments that could be designed to elucidate the effectiveness of different management approaches to the recolonization of bighorn sheep. In fact, many of the potential treatments have been conducted in a non-experimental fashion (see Bailey 1990 for a review). On the western slope of Colorado, the number of potential study sites for bighorn sheep research is high. The Dolores Canyon (west of Durango) has had several failed reintroduction efforts,

despite being classified as viable bighorn habitat. The Escalante, Dominguez and Roubideau canyons east of the Uncompahgre Plateau all have bighorn sheep. However, this population is currently suffering from a *Pasturella* outbreak (B. Watkins, personal communication). Finally, Colorado National Monument and Debeque Canyon are potential study sites. Because many of these issues take place at the population level, any study would be long-term and broad in scale. Although not explicitly detailed, conservation of bighorn sheep (especially desert bighorn sheep) is loosely prioritized in section S-2 of the CDOW strategic plan (conservation of native species). External funding for this type of research would be difficult to secure. While private groups are interested in bighorn restoration, the level of support needed to address these questions is likely greater than that which they can provide. In terms of logistical support, based on conversations with Division employees from local through the regional levels, interest and support for this research is high.

### **Impacts of Natural Gas and Oil Extraction on Deer and Elk.**

The impact of natural gas and oil development on deer populations in Colorado appears to be a cyclical issue driven by economics, political policy and current world affairs. Of the impacts that resource extraction can have on deer populations, the two of most immediate concern are space use patterns and population performance (Fig. 3). In terms of space use, deer behavior can shift on broad scales, fine scales or both. For instance, a broad scale shift might manifest itself in the form of animals vacating any area where development is occurring. A fine scale shift might manifest itself in the form of avoiding habitat types, but not abandoning areas of development. Little information for this type of impact has been published.

In face of the sparse literature pertaining to the impacts of development on deer and elk, there is information available that pertains to the impacts of development on caribou. Behavioral adaptations similar to those mentioned above have been documented for caribou in response to resource extraction in the arctic (Cameron et al. 1992, Nellemann and Cameron 1998, Dyer et al. 2001, Nellemann et al. 2003). Despite documented shifts in caribou distribution and density, there has been no documentation of negative population level impacts caused by resource extraction. In fact, the number of caribou in the Central Arctic caribou herd increased from 5,000 to 20,000 during oil-field development (between 1975-1997, Cronin et al. 2000). Similarly, while calving caribou and cow/calf pairs have been observed to avoid roads, a depression of calf survival was not reported (Nelleman and Cameron 1998). While space use behaviors are important, the DOW is primarily concerned about mule deer population performance. Of the population parameters that could potentially be impacted, fawn survival is the most sensitive to disturbance and is subsequently the key parameter for monitoring. Of additional note, measuring changes in the proximate physiological factors that lead to depressed fawn survival may be an avenue for exploring the impacts of resource extraction if monitoring fawn survival is unrealistic.

Within the history of Colorado, the impacts of natural resource extraction on wildlife is a subject that has not been ignored. During the 1980's, the U.S. Department of Energy funded research on deer survival on the CA and CB tracts of the Naval Oil Shale Reserve in northwestern Colorado. This research was to be extended to include the impacts of oil-shale development. However, due to financial limitations in the extraction process, development never progressed beyond the initial phase (G. White, personal communication). A pilot study addressing the impacts of natural gas drilling on deer space-use is currently underway in southern Colorado (S. Wait, personal communication). Based on what is known about the current distribution of development, there are essentially two potential study areas in Colorado. There is interest in studying these impacts along the I-70 corridor where gas pad density approaches 1 per 20 acres. Development for natural gas extraction is also either occurring or proposed for the Roan plateau, as well as the Mamm and Divide Creek basins. Unfortunately, the current density of extraction platforms in the Mamm and Divide Creek basins is too high for a controlled experiment. The feasibility of conducting research on the Roan Plateau is unknown. Based on public sentiment reflected in the news media, development on the Roan Plateau is hotly contested. In order for this study to be accomplished,

the Division would likely be placed at odds with this public sentiment due to the need for intense development as a treatment effect. Away from the I-70 corridor, development is also underway in southwestern Colorado (east of Durango, see above). This area likely offers the greatest possibility for advanced research on this issue due to the ongoing dialogue between management agencies, and the advanced stages of research activity. Additionally, because development is progressing at a slower rate in this region, and because much of the proposed development is on the Southern Ute Indian Reservation, there is potential for conducting experimental research.

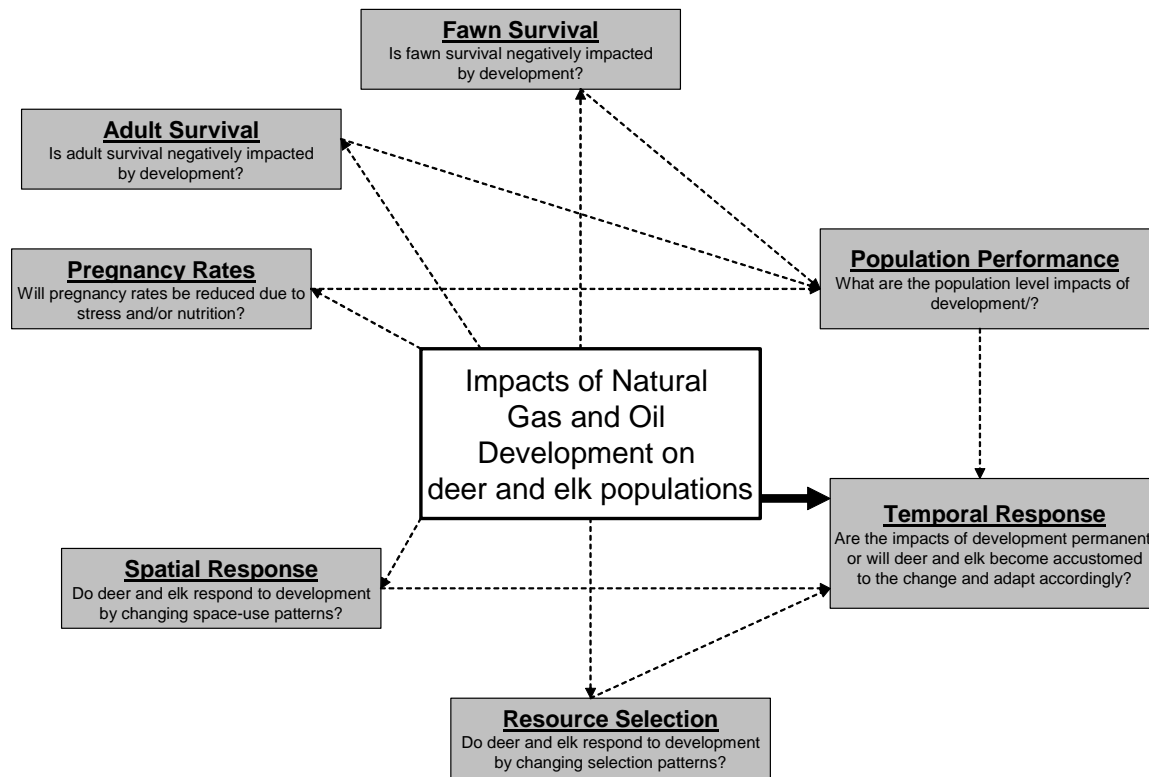


Figure 3. Conceptual model concerning the impacts of oil and gas development on deer and elk populations, with emphasis on potential impacts (gray boxes) and research possibilities (dashed lines).

From a greatly simplified viewpoint, natural resource extraction can be broken into two phases. Phase one is primarily composed of the building of infrastructure (i.e. road building, pipeline building, drill pad leveling, pump installation, etc.). The subsequent phase two is composed of steady state resource extraction (Fig. 4). In terms of impacts on deer, phase one is likely to have a strong, negative impact that is relatively short-lived. Infrastructure construction is typically high intensity and may be accompanied by a shift in space use behavior of deer (the longevity of this shift is open to debate). Phase two is typified by the physical extraction of the resource, a highly mechanized process that could require very little human presence on a daily basis. Due to the longevity of phase two, it is likely to have the longest lasting impact on deer (though the impact itself may be more subtle). However, the above described progression of development is an oversimplified scenario of how events could take place. Numerous uncertainties affect the rate of development, many of which are tightly linked to current governing policy and the overall state of the economy. For instance, it is possible that due to economic hardship or due to inefficiencies in the resource refining process, development would be aborted before the completion of phase one (Fig. 5). This sequence of events are similar to those that took place during the 1980's on the CA and CB tracts of the Naval Oil Shale Reserve (G. White, personal communication).

Conversely, it is also possible that regulatory policy could be relaxed during phase one of development. Thus, instead of progressing into phase two, phase one would be closely followed by a period of even more intense development (Fig. 6). The most likely scenario for the rate of development over the next 10-15 years, however, is a merger of both of these last two possibilities (i.e. development that is marked by peaks and valleys driven by the policy and economic events of the time, Fig. 7). As such, any study would have to accommodate a highly unpredictable and sporadic development pattern (Fig. 8).

As mentioned above, published experimental research on the impacts of resource extraction has been scarce. The reasons for this can largely be condensed to three primary problems: 1) the lack of experimental control, 2) the necessity for long-term commitment, and 3) cost and logistics.

For experimental research to occur, the needed approach would be a treatment/control study design. Due to the fact that the natural process variation of mule deer fawn survival ranges between 0.04 and 0.81 (Unsworth et al. 1999) and to the longevity of this study, a pre/post experimental design would not provide meaningful results. However, through a treatment/control design, the confounding effects of this annual variation would be eliminated. Thus, the issue turns to maintaining clean control/treatment study areas and clean treatment affects. The criteria for a control study area include: 1) quality deer winter range similar to the treatment area (i.e. similar geographic, topographic and botanical composition), 2) close proximity to the treatment study area, such that annual weather patterns are shared between the treatment and control areas, 3) being located far enough from the treatment study area that development activities do not influence deer on the control site, 4) having no pre-existing development, and 5) remaining free of on site and nearby resource extraction/exploration during the 10-15 years covering the experiment. The criteria for an adequate treatment study area are equally complex. In addition to the underlying criteria for a control site, a treatment study area would need: 1) an absence of any pre-existing development, 2) a "phase one" development schedule that is not subject to change (regardless of political, social or economic factors), and 3) a "phase two" period of steady-state extraction and maintenance that is also void of further mining and exploration. Despite great efforts, these criteria would be difficult to meet. Mining companies cannot afford to slow the extraction process if the allowable rate increases, and likewise, they cannot afford to continue pumping if it isn't economically feasible. These economic and social factors that drive production are outside the control of DOW, DNR, high level political officials and the mining companies themselves.

Long term commitment is a factor that plagues all long term research programs. However, a failed commitment to see an experiment on oil/gas development to completion would provide very little in terms of knowledge. For many long term research projects the delivery of a treatment occurs in the early stages, only to be monitored in the later years. The treatment in a study concerning oil/gas development would be two-fold, i.e. the treatment (or lack thereof) would be applied every year for 10-15 years. Willingness to maintain this program would need to persist in light of the political and economic changes that will inherently occur. Policy regulating natural resource extraction could become more stringent or more relaxed (discussed above). If the economic potential of development is not realized, long term commitment is not feasible.

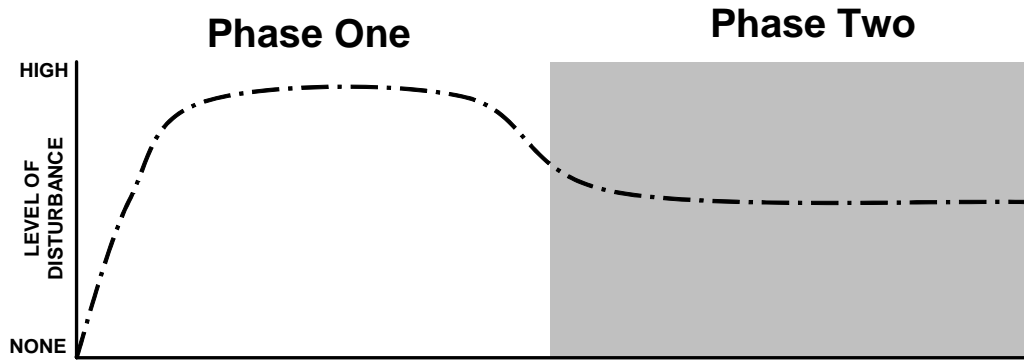


Figure 4. A conceptual diagram showing the ideal separation between phases of natural resource extraction and subsequent development. Phase one is typified by high intensity, infrastructure construction. Phase two is typified by lower intensity, higher longevity, extraction processes.

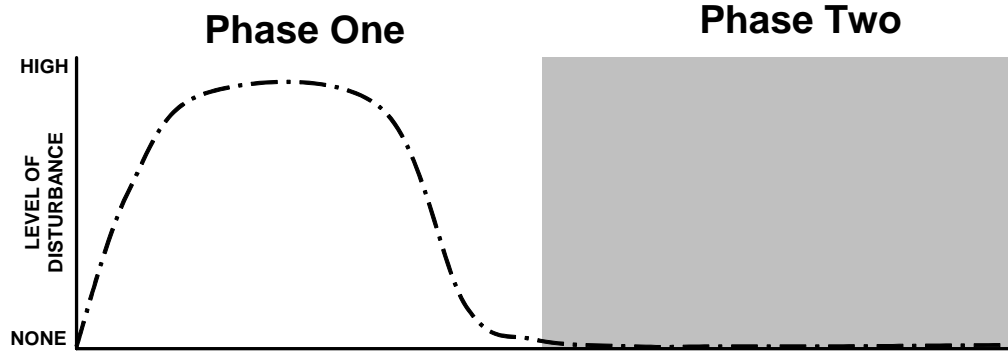


Figure 5. A conceptual diagram showing potential separation between phases of natural resource extraction and subsequent development. As opposed to ideal conditions, phase one could be cut short due to changes in the economy or changes in regulatory policy.

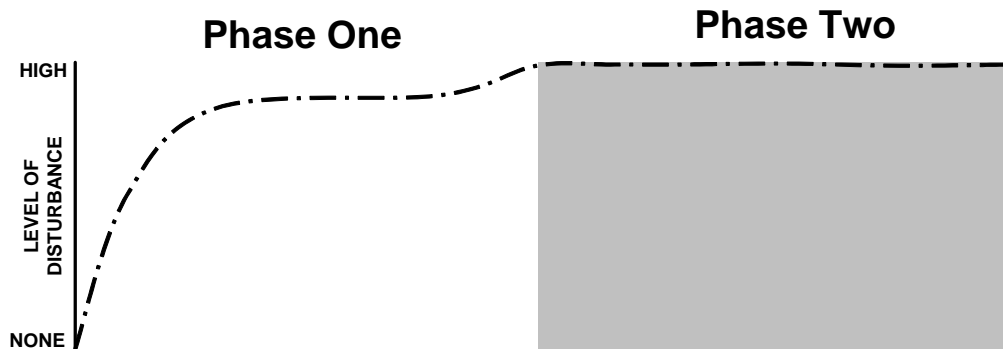


Figure 6. A conceptual diagram showing potential separation between phases of natural resource extraction and subsequent development. As opposed to ideal conditions, phase one could be followed immediately by further development, a result of economic incentives or relaxation in regulatory policy.



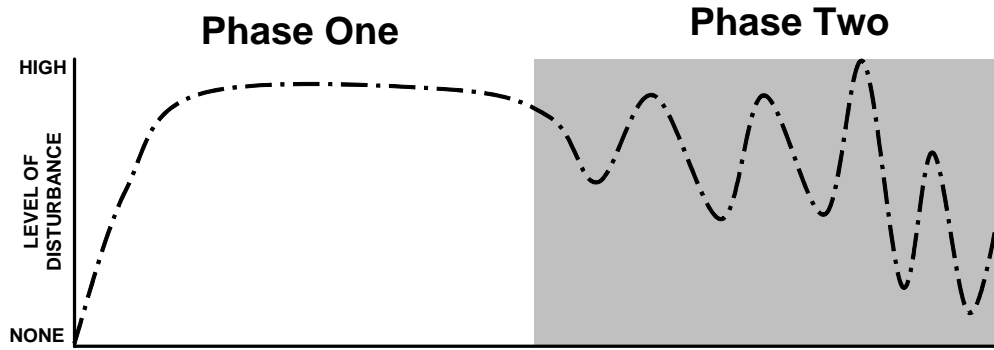


Figure 7. A conceptual diagram showing likely separation between phases of natural resource extraction and subsequent development. As opposed to ideal conditions, phase one will probably be followed by highly fluctuating stages of further development and extraction. The peaks and valleys of this scenario would be driven by normal economic variation and changes in regulatory policy.

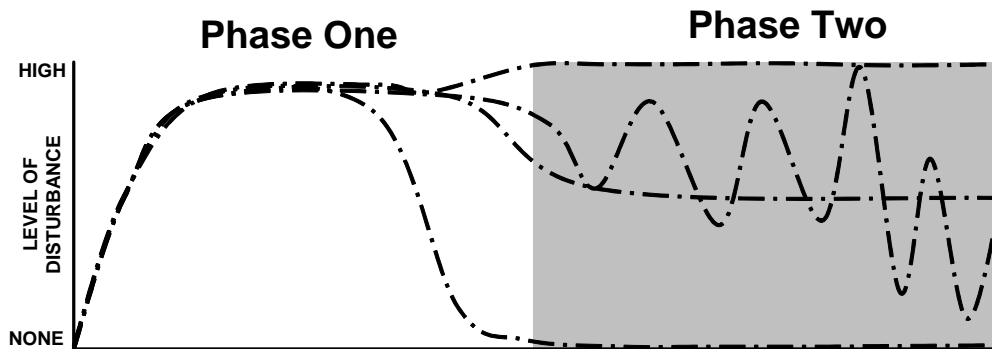


Figure 8. A diagram showing the potential development patterns that could be encountered during experimental research on the impacts of resource extraction. The variability shown in phase two is an uncontrollable product of changing economic patterns and changing regulatory policy. The lack of control highlighted by this figure demonstrates the underlying reason that experimental research on this issue is impossible.

Cost, the third factor, is also an obstacle encountered during any study. Based on power calculations from other mule deer fawn survival studies, a sample size of 40 marked fawns per study area was deemed necessary to detect a 15% change in fawn survival (Bishop 2000). However, in this example, experimental design increased the power of detecting this difference in excess of 80% because annual results could be pooled over consecutive years. A compiling of consecutive years is not possible when studying the impacts of resource extraction because a carry over effect is present and the impact of development compounds each year. Thus, reductions in fawn survival would need to be captured on a yearly basis. A preliminary power analysis ( $\alpha=.05$ ,  $\beta=.20$ ) using estimates of fawn survival of  $\mu=.444$  and  $SD=.217$  (Unsworth et al. 1999) indicated that 60 marked fawns per study area would be needed to have an 80% chance of detecting a 15% change in survival. However, a 15% change in survival is unrealistic in the face of published literature. A more likely expectation is in the realm of a 5%-10% decrease. Thus, more appropriate sample size estimates are between 125 (to detect a 10% change) and 480 (to detect a 5% change) for each of 2 study areas (annual sample sizes would thus range from 250-960 individuals).

Due to a lack of dialogue with personnel from natural gas and oil companies, the DOE, the Colorado Oil and Gas Conservation Commission and the Southern Ute Indian Reservation, the possibility of external funding for this type of research is unknown. Based on past efforts of these entities, it is believed that some level of financial support is possible. The issue of development is addressed by high priority achievement H-1.3 of the CDOW Strategic Plan (2002-2007). While the impacts of natural gas and oil extraction development, per se, are not addressed, they do qualify as a developmental issue of concern.

## SUMMARY

Mule deer/elk interactions, bighorn sheep translocation, and impacts of natural gas and oil extraction are all topics that present suitable and needed research investigations. At this time, moving forward on mule deer/elk interactions appears to be the most reasonable course of action. Cooperative commitments between industry and the State of Colorado appear needed before research could be initiated to meaningfully assess the impacts of natural gas extraction on mule deer populations

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Prepared by

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Eric J. Bergman, Wildlife Researcher



### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. \_\_\_\_\_ : Mammals Research  
Work Package No. 3740 : Wildlife Diseases  
Task 1 : Chronic Wasting Disease in Mule Deer  
Research and Development  
Federal Aid Project: N/A :

Period Covered: July 1 2003 through June 30, 2004

Author: Michael W. Miller and L. L. Wolfe

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### ABSTRACT

We continued conducting research on various aspects of chronic wasting disease (CWD) epidemiology and management. Here, we report progress in ongoing and recently-completed work. Studies focused on improving and expanding surveillance in free-ranging populations, understanding and modeling transmission mechanisms, identifying ecological and anthropogenic factors that may influence epidemic dynamics, and evaluating and applying alternative diagnostic and control strategies. In addition to preliminary findings reported here, 12 original studies and review articles were published during this segment; citations are appended to the report.

# **JOB PROGRESS REPORT**

## **CHRONIC WASTING DISEASE IN MULE DEER RESEARCH AND DEVELOPMENT**

Michael W. Miller and L. L. Wolfe

### **INTRODUCTION**

We continued conducting research on various aspects of chronic wasting disease (CWD) epidemiology and management. Some parts of this work were conducted in collaboration with investigators at Colorado State University, the University of Wyoming, and elsewhere. Specific projects were supported with various combinations of funds from the Colorado Division of Wildlife (CDOW), Federal Aid in Wildlife Restoration Project W-153-R, the U.S. Department of Agriculture (APHIS/VS), the U.S. Department of Interior (USGS/BRD), and National Science Foundation/National Institutes of Health (NIH) Grant DEB-0091961.

### **METHODS**

Our work on CWD is both multidisciplinary and multifaceted, but broadly falls under the topics of “epidemiology and management” or “pathogenesis and diagnosis”. For simplicity, we describe progress under those headings below.

#### **STUDIES OF CWD EPIDEMIOLOGY & MANAGEMENT**

We continued or initiated studies related to surveillance, transmission mechanisms, epidemic trend forecasting, potential host range and strain variation, risk factors, and management tools and feasibility as aids to understanding and controlling CWD in free-ranging deer and elk in Colorado.

Statewide surveillance: Surveillance for CWD continued throughout Colorado to determine the extent of distribution, to estimate prevalence in affected areas, and to monitor prevalence trends. Surveillance methods were as described elsewhere (Miller et al., 2000, *J. Wildl. Dis.* 36:676–690; Miller & Williams, 2002, *Vet. Rec.* 151:610–612; Hibler et al., 2003, *J. Vet. Diag. Invest.* 15:311–319).

In addition to reporting of annual survey findings, we also analyzed cumulative surveillance data to examine the potential influences of demographic, spatial, and temporal factors on observed prevalence patterns.

We also began exploring ways of improving the efficiency of our CWD surveillance program. Since 1996, tissue samples have been collected from deer killed in vehicle collisions throughout Colorado as part of our monitoring program for detecting CWD in free-ranging populations. We estimated CWD prevalence among vehicle-killed mule deer statewide and compared this to estimated CWD prevalence among mule deer sampled in the vicinity of these collision sites to determine if CWD-infected individuals were more vulnerable to vehicle collisions than otherwise healthy deer.

Transmission mechanisms: We summarized findings on empirical evidence of direct and indirect CWD transmission and the relative importance of these mechanisms in epidemic dynamics.

Modeling epidemic dynamics in captive mule deer: We continued analyses of 26 years of data (1974–2000) from CWD epidemics at CDOW’s Foothills Wildlife Research Facility to evaluate strength of evidence for a set of candidate models involving indirect and/or direct transmission, with and without latency periods. Estimates of transmission rates derived from these models will provide an upper bound

on what could be expected in wild populations and will guide construction of candidate sets for modeling those populations.

Host range and strain variation: We continued a series of experimental studies in cattle, fallow deer, and mountain lions to explore potential host range of CWD after intense but natural exposure; these experiments compliment ongoing surveillance for evidence of infection in species not known to be natural hosts of CWD, including moose and mountain lions.

We also completed work looking for evidence of strain variation in CWD agent from various deer sources using domestic ferrets as a laboratory model.

Effects of land use on prevalence: We summarized findings on the apparent effects of urban vs. nonurban land use patterns on CWD prevalence in mule deer.

Selective predation upon infected mule deer: We continued a study comparing prevalence of CWD among puma-killed mule deer to prevalence among mule deer harvested or randomly culled by humans within home ranges of collared mountain lions to assess whether predation is selective for CWD-infected mule deer. Methods were as described previously (Miller and Wolfe, 2003, Work Package 3430, Task 7410, Progress Report, Colorado Division of Wildlife, Ft. Collins). A total of eight adult mountain lions have been collared, resulting in 39 collared cat months between February 2001 and May 2004. Sampling of predator-killed deer is ongoing.

Influence of trace minerals on susceptibility: We continued two independent studies to investigate the potential influence of trace minerals on CWD susceptibility. In a retrospective study, we completed analyses of archived tissues to compare tissue levels of copper (Cu), molybdenum (Mo), and manganese (Mn) in mule deer infected with CWD to levels in apparently uninfected deer from the same geographic area.

We also continued an experiment to examine the effect of Cu supplementation on CWD susceptibility in white-tailed deer, wherein we are comparing the natural infection rate and course of CWD in captive deer receiving a sustained-release oral Cu supplement to the rate and course in unsupplemented controls residing in the same paddock.

Genetic influences on susceptibility: We continued collaborating with investigators from the University of Wyoming (UWYO) in studies of genetic influence on CWD susceptibility in mule deer. The main objective of ongoing UWYO research has been to search the DNA sequence of the PrP encoding region in exon 3 of the *Prnp* gene of mule deer for genetic variations that may influence occurrence of naturally acquired CWD. Recent analyses included samples from 529 free-ranging mule deer from four Colorado DAUs (326 from D-10, 63 from D-19, 71 from D-9, and 69 from D-7). Total genomic DNA was extracted from each sample and the PrP coding region from each deer genome was amplified by polymerase chain reaction (PCR). Genotyping was done using commercial sequencing or a simple restriction enzyme digestion (J. E. Jewell, unpublished data) of the PCR amplified PrP gene.

Preventive therapies: We collaborated with investigators from the Rocky Mountain Laboratories, NIH, to conduct a pilot study evaluating safety and efficacy of three prospective therapies for preventing CWD in mule deer. Twenty hand-raised mule deer were randomly divided into groups of 5 and assigned to receive a candidate therapy (coded PP, TA, or TC) or no therapy (control). We administered therapies continuously or 3× daily depending on the drug used; administration began 14 days before inoculation, and continued for 14 days after challenge. All groups received pelleted feed and alfalfa hay from the

same source. We used a novel oral inoculation method (M. W. Miller & L. L. Wolfe, unpublished data) for experimental challenge. We collected tonsil biopsies (Wolfe et al., 2002, *J. Wildl. Manage.* 66:564–573) from controls about 4 mo post inoculation (PI) and principals about 5 mo PI to assess efficacy of respective therapies in preventing CWD in mule deer.

Evaluation of an urban CWD management strategy: We completed an assessment of the feasibility of “test and cull” as an approach for managing CWD in urban habitats, and continued a 5-year study to evaluate the efficacy of this approach in reducing CWD prevalence among urban mule deer. During October 2003–May 2004, we again captured and tested free-ranging mule deer, and marked them with timed-release radiocollars in urban areas throughout Estes Park; our work was complimented by a parallel, coordinated effort by the US National Park Service (NPS) to capture and test deer inside Rocky Mountain National Park (RMNP). The collective annual goal was to test  $\geq 50\%$  of the adult mule deer residing the Estes Park population unit (Conner and Miller, 2004, *Ecol. App.* in press); target sample sizes (52 adult males and 153 adult females) were estimated based on a mark-resight inventory conducted in December 2003. Field methods were as previously described (Wolfe et al., 2004, *Wildl. Soc. Bull.* in press). In addition to the primary goal of assessing the efficacy of test and cull as a management strategy, data gathered in the course of this study will also be useful in improving our understanding and modeling of the influences of urban landscapes on CWD epidemiology.

#### STUDIES OF CWD PATHOGENESIS & DIAGNOSIS

We continued or initiated studies related to pathogenesis in natural hosts and live-animal diagnostic test refinement and evaluation as aids to improving approaches for CWD surveillance and diagnosis in free-ranging deer and elk in Colorado.

Pathogenesis in natural host species: We completed our work studying the pathogenesis of CWD in white-tailed deer after oral inoculation with infectious, conspecific brain tissue. This study will complement studies documenting CWD pathogenesis in mule deer and elk that already have been completed.

Evaluation of antemortem diagnostic techniques: We continued working to refine and evaluate tonsil biopsy techniques for diagnosing CWD in live animals. In light of our continued success in applying established techniques (Wolfe et al., 2002, *J. Wildl. Manage.* 66:564–573), we have continued using tonsil biopsy to gather data for field studies and epidemiological investigations. We also began using tonsil biopsy IHC as diagnostic benchmark for evaluating other candidate tests for diagnosing CWD in live animals.

In conjunction with ongoing studies on CWD transmission, we evaluated a candidate rapid test developed by Prion Developmental Laboratories, Inc. (PDL), modified for potential use under field laboratory conditions (J. E. Jewell, unpubl. data). Initial evaluation of this test on biopsy-sized pieces of tonsil tissue collected postmortem from culled mule deer revealed that sensitivity (about 80%) was near the lower limit of acceptability for field use. Modifications to improve sensitivity were made, and subsequently we evaluated sensitivity of the PDL test under conditions simulating those anticipated in field applications using tonsil biopsies collected from captive mule deer naturally infected with CWD. Tissue samples collected via tonsillar biopsy (Wolfe et al., 2002, *J. Wildl. Manage.* 66:564–573) were examined within 10 min of collection via the candidate PDL test; details of laboratory techniques were proprietary. Laboratory equipment and conditions simulated those that we anticipated would be available at a field site. Paired biopsies were collected from infected and uninfected deer ( $n = 16$ ); we randomly assigned one of each pair to PDL and the other to immunohistochemistry (IHC) evaluation. Biopsies were processed, test reactions evaluated, and deer categorized as CWD-positive or -negative based on observed reactions; laboratory personnel were unaware of the infection status of sampled deer. Time



from sample collection to reporting of test result was recorded for each biopsied deer. Sensitivity (estimate, 95% CI) of the PDL test was calculated, using IHC as the reference standard. We compared the proportion of positive deer detected by the PDL test to results from IHC using a one-sided Fishers exact test; we used  $\alpha = 0.1$  for all analyses. In addition, the mean reporting time and range of reporting times was calculated for use in assessing the utility of the PDL test under anticipated field conditions.

In conjunction with ongoing studies on CWD prevention, we also reevaluated nictitating membrane (also called the “third eyelid”) biopsy as an approach for detecting CWD in live mule deer. We used a modified technique devised for domestic sheep (S. Bender, unpublished data) to identify lymphoid tissue on the nictitating membrane and adjacent conjunctiva, then collected biopsies using established techniques (O’Rourke et al., 1998, Vet. Rec. 142:489-491). We sampled both eyes of 11 mule deer experimentally infected with CWD and known to be tonsil biopsy positive. Nictitating membrane biopsies were evaluated by IHC using published methods (O’Rourke et al., 1998, Vet. Rec. 142:489-491; O’Rourke et al., 2002, Clin. Diag. Lab. Immunol. 9:966-971). We calculated the proportion ( $\pm$  95% CI) of usable nictitating membrane biopsies, as well as the sensitivity ( $\pm$  95% CI) of nictitating membrane biopsy IHC for CWD diagnosis using tonsil biopsy IHC as the reference; criteria for regarding this nictitating membrane biopsy technique as potentially useful in diagnosing CWD in mule deer were  $\geq 90\%$  of samples containing usable lymphoid tissue and estimated sensitivity  $\geq 95\%$ .

We also collaborated in a second study to evaluate a prospective rapid blood test (GeneThera, Denver, CO) for diagnosing CWD in live deer. A total of 10 blood samples from tonsil biopsy-positive, captive mule deer were collected by GeneThera representatives using collection materials and protocols provided by the laboratory; samples were immediately taken to their laboratory for evaluation. By previous agreement, the status of sampled animals was known to GeneThera personnel prior to blood collections.

## RESULTS AND DISCUSSION

### STUDIES OF CWD EPIDEMIOLOGY & MANAGEMENT

**Statewide CWD surveillance:** The CDOW sampled over 15,000 deer and elk harvested or culled in northern Colorado and other select locations, as well as smaller numbers of deer and elk submitted as clinical suspects. Surveillance revealed two previously undetected CWD foci in mule deer, one on the Grand Mesa (DAU D-51) and the other in Colorado Springs (DAU D-16). Survey results will be posted on the Division’s CWD web page (). Surveillance data also will be used to augment an existing database that is the foundation for ongoing analyses and modeling of temporal and spatial aspects of CWD epidemiology, as well as for evaluating responses to management.

In addition to reaffirming the spatial heterogeneity among wintering mule deer subpopulations observed previously (Miller et al., 2000, J. Wildl. Dis., 36:676–690; Conner & Miller, 2004, Ecol. App., *in press*), our analyses revealed marked differences in CWD prevalence by sex and age groups, as well as clear local trends of increasing prevalence over a 7-yr period. CWD prevalence differed ( $P < 0.0001$ ) by age (yearling vs. adult), sex, and geographic area at two different spatial scales (game management unit [GMU] or population unit winter range), and increased over time at both geographic scales (GMU:  $\beta = 0.064$ , 95% CI = 0.009–0.119,  $P = 0.0219$ ; population unit:  $\beta = 0.263$ , 95% CI = 0.134–0.399,  $P < 0.0001$ ). Disease status (positive or negative) was not independent of age for males ( $n = 947$ ,  $df = 3$ ,  $\chi^2 = 459$ ,  $P < 0.0001$ ) or females ( $n = 549$ ,  $df = 4$ ,  $\chi^2 = 71$ ,  $P < 0.0001$ ). For both sexes, prevalence peaked in the 4–6-yr old age class, with the largest increase occurring between the 2–3-yr-old and 4–6-yr-old age classes. This differential was larger for males: prevalence rose from 5.9% (95% CI = 4.9–6.8) among 2–3-yr-olds to 19.4% (95% CI = 12.1–26.7) among 4–6-yr-olds ( $P = 0.0002$ ); for the 4–6 yr age class, prevalence among males (19.4%) was 2.7 $\times$  greater ( $P = 0.0006$ ) than among females (7.2%).

Demographic, spatial, and temporal factors all appear to contribute to the marked heterogeneity in CWD prevalence in endemic portions of northcentral Colorado. These factors likely combine in various ways to influence epidemic dynamics on both local and broad geographic scales. A manuscript describing our findings is in review for publication in the *Journal of Wildlife Diseases*.

Sampling of vehicle-killed mule deer may be exploited in increasing the efficiency of surveillance programs designed to detect new foci of CWD infection and direct management actions; however, this differential vulnerability also may bias prevalence estimates in natural populations when data from vehicle-killed deer are included in calculating such estimates. Overall CWD prevalence was 1.66× higher in vehicle-killed deer; prevalence among vehicle-killed deer was 0.101 (95% confidence interval [CI] = 0.064–0.139) compared to 0.061 (95% CI = 0.051–0.072) prevalence among mule deer harvested, culled, or biopsied within 3 km of collision sites. The probability of detecting a CWD-infected, vehicle-killed deer, given that at least one other CWD-infected deer had been detected within a 3 km radius of the vehicle-kill site, was 16.7%. Our data suggest increased susceptibility of CWD-infected individuals to vehicle collisions. Evidence of increased susceptibility to vehicle collisions also may aid in understanding vulnerability of CWD-infected individuals to other forms of death, particularly predation. A manuscript describing our findings is in review for publication in the *Journal of Wildlife Diseases*.

Transmission mechanisms: Manuscripts describing our findings on the relative importance of animal–animal transmission of CWD and on the relative contributions of live animals, contaminated environments, and infected carcasses to CWD transmission were accepted for publication and subsequently published in peer-reviewed journals (see Appendix for citations).

Modeling epidemic dynamics in captive mule deer: Preliminary analyses suggest that indirect transmission models best represent epidemic data; moreover, our model selection results align well with independent empirical findings on CWD transmission mechanisms (Miller et al., 2004, *Emerg. Inf. Dis.* 10:1003–1006). We will continue refining candidate models before making final comparisons and parameter estimations. Findings should be of use in refining epidemic models of CWD in free-ranging mule deer populations.

Host range and strain variation: Cattle (n = 11) living in paddocks with naturally-infected mule deer remained healthy through 7 years of exposure; in contrast, only 1 of 12 mule deer introduced into these same paddocks in 1997 is still alive. Our results are consistent with data from cell-free conversion (Raymond et al., 2000, *EMBO* 19:4425–4430) and intracerebral (IC) challenge (Hamir et al., 2001, *J. Vet. Diag. Invest.* 13:91–96) studies that suggest the probability of natural susceptibility to CWD in cattle is extremely low. Similarly, neither signs nor postmortem evidence of infection have been observed in fallow deer (n = 24) exposed to infected mule deer for ≤3.5 years, and mountain lions (n = 3) consuming carcasses of CWD-infected deer and elk for >2 years also have remained healthy. No evidence of infection has been observed in moose, mountain lions, or cattle examined via ongoing surveillance.

Clinical signs and postmortem findings consistent with CWD in ferrets were observed in four of five IC-inoculated with tissue from infected deer, but were not observed in the free-ranging white-tailed deer or control groups. Incubation and incubation periods were consistent among affected groups. Preliminary assessment of Western blots (WB) revealed no apparent differences in glycosylation patterns among WB-positive ferrets, and no evidence of infection in the unaffected white-tailed deer or control groups.

Effects of land use on prevalence: Urban land use appears to affect CWD prevalence: rates were higher in developed areas and among male mule deer, suggesting anthropogenic influences on the

occurrence of CWD. We also observed relatively high variation in prevalence across three study sites (Estes Park, Horsetooth Mountain, Glacier View Meadows), suggesting that spatial patterns may be influenced by other factors operating at a broader, landscape scale. Our results suggest that multiple factors, including changes in land use, differences in exposure risk between sexes, and landscape-scaled heterogeneity, are associated with CWD prevalence in north-central Colorado. A manuscript describing these findings is currently “in press” (see Appendix for citation).

Selective predation upon infected mule deer: Our work continues from a pilot study conducted to evaluate available global positioning system (GPS)-based telemetry collars for use in this sampling application. Three collar styles have been deployed, and we are continuing to test and evaluate this new technology; aside from our main objective of data gathering related to CWD ecology, evaluation of this technology should be a substantial contribution to future studies of predator-prey relationships. We have detected and examined over 85 kill sites from radio-collared mountain lions and successfully sampled tissues from 28 sites where adult mule deer carcasses were present; we also have collected 17 samples opportunistically from mule deer killed by mountain lions that were not radio-collared. We will continue capturing mountain lions to reach the objective of six to nine collared cat years, and will continue sampling carcasses of lion-killed mule deer to reach our target sample size ( $n = 157$ ). We also will continue refining our monitoring approach to ensure that we find kill sites quickly enough to retrieve a suitable tissue sample to test for CWD. Whether target sample sizes can be attained in the time planned for this work remains to be determined.

Influence of trace minerals on susceptibility: Both studies are well underway. Laboratory analyses of retrospective samples are complete, and data analysis is underway. Experimentally- treated and control deer are being sampled on a regular schedule, but laboratory analyses are incomplete.

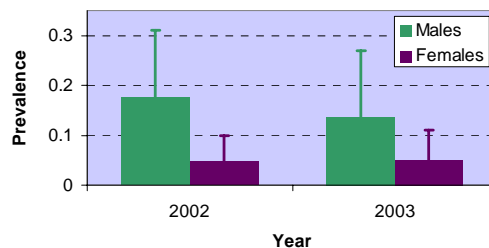
Genetic influences on susceptibility: Only four codons in the open reading frame of the *Pnprp* gene exhibit variation in mule deer, and only one of the four results in a change in the final version of PrP (Brayton et al., 2004, *Gene* 326:167–173; J. E. Jewell, unpublished data) -- this is a change from the amino acid serine (S), the high frequency allele, to phenylalanine (F) at codon 225. Preliminary results showed that estimated frequency of F allele occurrence in gene pools was similar in Colorado DAUs with (D-10: 0.095;  $n=652$ ) and without endemic CWD (D-19: 0.111;  $n=126$ ). However, F225 was not detected in the genomes of CWD-infected deer from D-10 ( $n = 50$ ), and the F225 gene frequency was lower than in uninfected D-10 deer ( $0.1$ ;  $\chi^2 = 4.6$ ,  $P < 0.05$ ). We observed a similar pattern of low F225 gene frequency among mule deer infected with CWD after experimental exposure via direct and indirect routes (Miller et al., 2004, *Emerg. Inf. Dis.* 10:1003–1006). Whether F225 affects truly affects CWD susceptibility or transmission in mule deer remains to be determined, and is the subject of continued investigation.

Preventive therapies: All 4 control deer that survived to 4 mo PI showed evidence of PrP<sup>CWD</sup> accumulation in tonsil biopsies collected ~4 mo PI. Unfortunately, all but 2 of the 15 treated deer also showed PrP<sup>CWD</sup> accumulation in tonsil biopsies collected ~5 mo PI; the 2 apparently uninfected deer were both from the same treatment group (PP), but overall infection rate did not differ ( $P = 0.28$ ) from control. We will continue following these deer to examine potential differences in post-exposure survival that could be attributable to therapies, and to further document the outcomes of the alternative inoculation method used. We also plan to continue this work if other candidate therapies become available.

Evaluation of an urban CWD management strategy: Data from the 2002–2003 field season indicated that testing and culling mule deer in Estes Park could be done at rates needed to evaluate the efficacy of this approach in reducing CWD prevalence. A manuscript describing the results of our feasibility study is in preparation is “in press” for publication in the *Wildlife Society Bulletin*.

Because we were successful in reaching objectives for population-level testing, the 2002–2003 field season became year 1 of a 5-year study to evaluate the efficacy of “test and cull” as a CWD control strategy. In year 2 (2003–2004 field season), we captured and tested 44 adult ( $\geq 1.3$  yr old) male and 119 adult female mule deer in Estes Park. CWD prevalence was about 13.6% among males and 5% among females tested in

Estes Park (Fig. 1); although no clear evidence of a treatment effect has emerged (Fig. 1), it is probably unrealistic to expect measurable changes in prevalence after only 1 year of test and cull management. The combined efforts of CDOW and RMNP programs resulted in an overall testing rate of 63% of the deer wintering in the Estes Park vicinity, including about 90% of the estimated 103 male and 55% of the estimated 306 female deer in this population unit.



**Figure 2.** Chronic wasting disease (CWD) prevalence among male (teal bar) and female (plum bar) mule deer tested in Estes Park, Colorado, 2002–2004. Prevalence between years did not differ (Fisher exact test  $P \geq 0.4$ ). Vertical lines are upper 95% confidence limits on estimated prevalence.

## STUDIES OF CWD PATHOGENESIS & DIAGNOSIS

**Pathogenesis in natural host species:** White-tailed deer inoculated orally with about 2.5 g of brain tissue homogenate (containing about 15  $\mu\text{g}$  PrP<sup>CWD</sup>) developed clinical CWD and were euthanized in end-stage disease 16–30 mo postinoculation (PI). The clinical course in inoculated white-tailed deer was similar to that previously observed in mule deer inoculated with about 15  $\mu\text{g}$  PrP<sup>CWD</sup> from infected mule deer. Laboratory evaluations of tissues from both our white-tailed deer and mule deer pathogenesis studies are pending.

**Evaluation of antemortem diagnostic techniques:** Tonsil biopsy is a useful tool for estimating CWD prevalence in nonhunted mule deer populations. In addition to applications in the two field studies described here, the techniques we developed are being used in at least six other field studies of CWD epidemiology (WY, NM, WI, SD, NE, CO).

Although the PDL test showed considerable promise as a potential field test, assay performance will need to be improved before it can be incorporated into ongoing CWD research or management programs. We observed good assay sensitivity (1.0; 6/6), but relatively low specificity (0.7; 7/10); overall agreement with IHC was 0.64 (95% CI = 0.29–0.98). There appeared to be an unacceptably high number of “false positive” tests -- application in a low prevalence population (e.g., Estes Park) would likely lead to unnecessary culling of numerous healthy deer, and could erode public support for our field study. Consequently, the PDL test was not incorporated into the 2003–2004 field study in Estes Park.

The nictitating membrane biopsy technique provided a high proportion of usable samples: all 22 samples contained at least 1 lymphoid follicle and 12–16/22 (55–73%) samples contained  $\geq 9$  follicles. Unfortunately, IHC of nictitating membrane biopsies detected PrP<sup>CWD</sup> accumulation in only 2/22 biopsies, both from the same deer. Because estimated sensitivity (0.09; 95% CI 0.01–0.29) is inadequate, we cannot recommend incorporation of nictitating membrane biopsy IHC into any of our ongoing CWD research or management programs.

We remain unable to assess the reliability or repeatability of the “GeneThera test”. No test results were provided on the 10 blood samples from positive mule deer; instead, a company representative indicated that extractions from samples were unsuccessful, and that consequently tests could not be run.

This is our second unsuccessful attempt to obtain results from blood samples submitted to GeneThera for CWD testing. Until an evaluation of their test can be completed, we cannot recommend its incorporation into any of our ongoing CWD research or management programs.

## APPENDIX

### Publications arising from ongoing CWD work:

- Belay, E. D., R. A. Maddox, E. S. Williams, M. W. Miller, P. Gambetti, and L. B. Schonberger. 2004. Chronic wasting disease and potential transmission to humans. *Emerging Infectious Diseases* 10:977–984.
- Brayton, K. A., K. I. O'Rourke, A. K. Lyda, M. W. Miller, and D. P. Knowles, Jr. 2004. A processed pseudogene contributes to apparent mule deer prion gene heterogeneity. *Gene* 326:167–173.
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- O'Rourke, K. I., D. Zhuang, A. Lyda, G. Gomez, E. S. Williams, W. Tuo, and M. W. Miller. 2003. Abundant PrP<sup>CWD</sup> in tonsil from mule deer with preclinical chronic wasting disease. *Journal of Veterinary Diagnostic Investigation* 15: 320–323.
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- Williams, E., and M. Miller. 2003. Prions in the wild: CWD in deer and elk. *Microbiology Today* 30: 172–173.
- Wolfe, L. L., W. R. Lance, and M. W. Miller. 2004. Immobilization of mule deer with thiafentanil (A-3080) or thiafentanil plus xylazine. *Journal of Wildlife Diseases* 40: 282–287.

Prepared by \_\_\_\_\_

Michael W. Miller, Veterinarian



Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3440  
Project No. \_\_\_\_\_ : Wildlife Health Program  
Work Package No. 3740 : Wildlife Diseases  
Task \_\_\_\_\_ : Wildlife Disease Surveillance Technical and  
Laboratory Support  
Federal Aid Project \_\_\_\_\_ :

Period Covered: July 1 2003 through June 30, 2004

Author: L. A. Baeten

Personnel: K. Cramer, K. Green, K.A. Griffin, E. Knox, C. T. Larsen, M. W. Miller, L. L. Wolfe and  
D. Wroe

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### ABSTRACT

The Wildlife Health Laboratory (WHL) was initially created in 2002 to meet expanded needs for chronic wasting disease (CWD) surveillance throughout Colorado. WHL activities supported CWD epidemiology and management work, as well as various new and ongoing CWD research projects. In addition, the WHL has been able to meet demands for diagnostic and laboratory services related to other wildlife diseases that have come to the forefront of concern in the management of Colorado's wildlife resources.

# **JOB PROGRESS REPORT WILDLIFE DISEASE SURVEILLANCE TECHNICAL AND LABORATORY SUPPORT**

L. A. BAETEN

## **INTRODUCTION**

The Wildlife Health Laboratory (WHL) was created in 2002 in response to the H-1.1 objective of the Division's Strategic Plan. The purpose delineated in this objective is to "*aggressively research, identify, detect, contain and eliminate, where possible, diseases in free-ranging wildlife and captive wildlife that could negatively impact wildlife populations*". The WHL was developed to meet the expanded needs for chronic wasting disease (CWD) surveillance throughout Colorado. WHL activities supported CWD epidemiology, harvest testing and management work, as well as various new and ongoing CWD research projects. In addition, the WHL has been able to meet demands for monitoring, detection, and diagnostic laboratory services related to other wildlife diseases that have come to the forefront of concern in the management of Colorado's wildlife resources (i.e. West Nile virus, plague, Pasteurellosis, etc.).

## **SUMMARY**

### Statewide CWD surveillance:

The discovery of CWD in northwestern Colorado in January 2002 created a sudden demand for both more widespread surveillance and more rapid turnaround on laboratory results. The CDOW's CWD surveillance program was modified in 2002-2003 to decrease turnaround time (from initial submission to acquisition and posting of results), improve data collection and quality control.

The notable changes in 2003-2004 were the addition of an electronic data collection system that was used statewide for collection of field and laboratory data. The WHL staff was instrumental in helping to delineate system mechanics, provide testing and troubleshooting capabilities and assist with training efforts. Details of overall programmatic features and changes were described on the CWD-oriented CDOW web page (<http://wildlife.state.co.us/CWD/index.asp>); details of the efficiencies in the sampling and testing procedures are described below. Numerous state agencies have request demonstrations of this new system for possible implementation in their CWD surveillance programs.

During 2003-2004, the CDOW sampled 17,268 deer, elk and moose harvested or culled in northeastern Colorado and other select locations. Survey results were posted on the Division's CWD web page (<http://wildlife.state.co.us/CWD/index.asp>). The data generated provided annual CWD survey results. These data were added to the cumulative surveillance data that is the foundation for ongoing analysis and modeling of temporal and spatial aspects of CWD epidemiology, examining potential influences of demographics, as well as evaluating responses to management.

In an effort to improve surveillance efficiencies, tissue samples were collected from deer killed from vehicle collisions throughout the state. Prevalence data from this group were analyzed to determine if CWD-infected individuals were more vulnerable than otherwise healthy animals.

Moreover, the surveillance strategy and methods first devised and implemented in Colorado continue to serve as a model for developing national recommendations on CWD surveillance in free-ranging populations.



### CWD tissue handling and disposal:

The WHL staff prepared documents summarizing published literature on appropriate disposal methods for CWD infected tissues (incineration and chemical). These documents were used extensively for public information during the review process for the incinerator proposal for Wellington. The WHL supervisor worked with EPA and national veterinary diagnostic laboratory representatives to develop “Best Management Practices” when handling CWD infected materials.

### Research projects

The WHL lab staff provided technical and diagnostic support for the ongoing DOW research projects listed below. Major accomplishments and contributions for the WHL this fiscal year include: completion of the experimental phase of the “Molecular epidemiology of strain variations in CWD”; evaluation of antemortem diagnostics (described below); the addition of DNA extractions to the list of diagnostic capabilities (supporting the “Genetic influences study and several species conservation projects); initiation of the pilot study on biosolids and wastewater; preliminary results from studies looking at prion inactivation are ready for presentation; and extensive sample collections for the studies of prions in biological excretions and environmental samples.

1. Molecular epidemiology of strain variations in chronic wasting disease (CWD)
2. CWD host range studies
3. Selective predation upon CWD-infected mule deer
4. Trace mineral influences on CWD susceptibility
5. Genetic influences on CWD susceptibility
6. Evaluation of preventative therapies for CWD
7. Evaluation of antemortem diagnostic techniques for CWD
8. Detection of prions in environmental samples
9. Detection of prions in biosolids and waste water
10. Chemical inactivation of prions
11. Detection of prions in biological excretions
12. West Nile virus in black-tailed prairie dogs
13. Prevalence of CWD in ungulates killed via vehicle collisions
14. Evaluation of a recombinant plague vaccine in lynx
15. Invertebrate role in CWD transmission
16. Evaluation of FWRP diarrhea outbreaks
17. Uncompaghre fawn mortality

Evaluation of antemortem diagnostic technique for CWD: In a pilot study, the WHL evaluated the use of a lateral flow strip test (Prion Developmental Laboratories, Inc.) to determine its applicability for “live animal testing” in the field. Approximately 40 tonsil samples were used to assess the applicability of this diagnostic test under field conditions. The test kit procedures were manipulated to determine if modifications to the lymph node procedures could be used to accommodate tonsil tissue (and tonsil biopsy sized tissues). It was determined that the assay could be modified to work under field

conditions (i.e. roving lab). However, despite efforts to modify the test parameters, the sensitivity was not acceptable to pursue further field trials with this new diagnostic test system.

In addition, the WHL staff provide technical assistance to collaborators interested in archived tissues for ongoing research projects listed in the table below. The WHL staff accomplished this via additional sample collections from hunter harvest, culls and other DOW submissions. These samples are archived then aliquoted and shipped to the collaborators according to specific tissue requests.

#### Collaborative Agreements

IND/HPF/USCF	Brain	Transgenic mouse development/ host range studies
USDA/ARS	Brain	Strain typing, comparison to other TSE strains
NYSIBR	Brain	Transgenic mouse strains
PDL	Lymph nodes	Lateral flow strip test
CSU	Multiple tissues	Experimental transmission (ante and post mortem collection)
USDA/ARS	Eyelids, blood	CWD assay evaluations
NIH/RML	Multiple tissues	Evaluate strain variations
CWRU	Brain, lymph nodes	Transgenic mouse host strain study, cellular prion transport
NYSIBR	Urine	CWD assay evaluations
CSU	Brain	Effects of composting on prion inactivation
IDEXX	Lymph nodes	Validation of diagnostic assay
USU	DNA extracts (blood)	Epidemiology studies
RMNP	DNA extracts (blood)	Epidemiology studies
GeneThera	Blood	Antemortem assay evaluation

#### Wildlife Disease Surveillance:

The WHL performed necropsies to assist state wildlife managers and biologists in determining the cause of death for wildlife species including: deer, elk, bighorn sheep, mountain goats, bear, various avian species and rodents (See Table 1). This necropsy effort included support for two species conservation projects. The WHL provided technical and diagnostic support for the field projects listed below. This effort included biological sample collection, data collection, sample processing, diagnostic testing, archiving and/or distribution of samples.

1. Evaluation of diagnostic techniques for avian translocations
2. Disease surveillance for Prairie grouse restoration
3. Disease surveillance for Turkey translocation
3. Bighorn sheep translocations: Identification of *Pasturella spp.* strains
4. Identification of Johne's disease in BHS and RMG
5. Identification of lungworm larvae in BHS feces
6. Black-footed ferret restoration: carnivore sampling
7. Lynx restoration
8. Winter deer capture: mule deer survival monitoring
9. Elk Fertility control
10. Test and cull evaluation
11. CWD management culling
12. Foot hills wildlife research facility

West Nile Virus: The WHL established in-house testing for West Nile virus (WNV). Fifty-four carcasses were submitted as suspects for necropsy and testing during this fiscal year. Thirteen positives were identified. In conjunction with the DOW WNV testing performed at the WHL, tissue samples collected during necropsies were provided to CDC (Komar) for their use in experimental trials developing a new post mortem assay for WNV.

Avian Translocations: The WHL investigated alternative diagnostic testing for avian translocation projects. An in-house assay for *Mycoplasma (synoviae, gallisepticum, meleagridis)* was determined to be optimal for testing individual birds being translocated. The use of the ELISA assay for these serological tests minimized the cross reactivity effects that were experienced with diagnostic tests used previously. With the use of the ELISA, next-day releases were possible, therefore, decreasing individual stress levels and increasing survivability for birds moved in translocation efforts. The WHL staff provided technical and diagnostic support for three avian translocation projects during this project year (sharp-tailed grouse, turkey and ring-necked pheasant).

In combination with the diagnostic necropsy support, the WHL established a database to allow electronic review of these data over time. All historical diagnostic records were incorporated into the database during this fiscal year. To date, this database contains approximately 400 records. From the diagnostic reports database, the WHL prepared wildlife disease summaries for statewide distribution. The wildlife disease summaries for years 2002 and 2003 delineate animal mortality data by species, quarter and region. This data will assist wildlife veterinarians, managers and biologists in future wildlife disease events.

During this fiscal year, the WHL established an archive database which includes all of the historical samples collected since the initial establishment of the WHL in the 1990's. This database allows WHL staff to determine what tissues are available for use in research projects, delineates physical locations where various tissue samples can be found, tracks distribution of tissue samples and contains appropriate animal identification and specifications. At the end of the fiscal year, there were a total of 4,850 entries with approximately 300 of those added for the year.

Table 1: Diagnostic Support

Species	Necropsies	Diagnostic Samples (collected, processed, archived)
Carnivore	3	40
Deer	30	385
Elk	10	4
Lynx	0	106
Other avian species (WNV)	36	36
Other ungulate	18	107
Prairie grouse	7	22
Small game	10	50
Small mammals	15	35
Total	126	745

Training sessions:

The WHL has provided multiple training sessions for CWD sample collection. The attendees included CDOW employees as well as federal employees from the Rocky Mountain region. In addition, the WHL staff assisted with training sessions for DWM trainees in necropsy techniques, darting and sample collections.

Presentations:

The WHL staff made various presentations on wildlife disease to various groups including the Wildlife Society, USFWS, CDOW staff, black-footed ferret subcommittee, Colorado Wildlife Federation. The titles of the presentations were:

1. West Nile Surveillance in Colorado 2003
2. The impact of West Nile virus on wildlife populations
3. The significance of West Nile virus in prairie dogs
4. Common diseases in wildlife populations of Colorado

**APPENDIX**

Publications arising from WHL contributions to ongoing CWD work:

- Brayton KA, O'Rourke KI, Lyda AK, Miller MW, Knowles Jr. DP. A processed pseudogene contributes to apparent mule deer gene heterogeneity. *Gene* 326: 167-173.
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- O'Rourke KI; Zhuang D; Lyda A; Gomez G; Williams ES; Tuo W; Miller MW. Abundant PrP<sup>CWD</sup> in tonsil from mule deer with preclinical chronic wasting disease. *J Vet Diagn Invest* 2003 13: 320-323
- Powers BE, Hibler CP, Spraker TR, Miller MW. Large scale surveillance for chronic wasting disease: The Colorado laboratory experience. *Annual proceedings USAHA* 2004 pg 64.

Prepared by \_\_\_\_\_  
Laurie A. Baeten, Veterinarian

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. \_\_\_\_\_ : Mammals Research  
Work Package No. 3740 : Wildlife Diseases  
Task \_\_\_\_\_ : Pilot evaluation of GPS technology in chronic  
wasting disease prevalence and management at  
artificial feeding sites in urban areas.  
Federal Aid Project: N/A :

Period Covered: April 1 2003 through July 31, 2004

Author: Eric J. Bergman, Michael W. Miller and L. L. Wolfe

Personnel: M. Sirochman

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### ABSTRACT

A pilot study for assessing the utility of GPS technology in the evaluation of CWD prevalence and management in urban areas was designed is being implemented. Objectives of this pilot study are to:

- 1) Evaluate the utility of GPS radio collar technology in identifying artificial feed sites in urban settings,
- 2) Evaluate if there is evidence that artificial feed sites reduce the size of deer home ranges,
- 3) Evaluate if deer density is elevated at artificial feed sites, and
- 4) Evaluate if CWD prevalence is higher at artificial feed sites

**JOB PROGRESS REPORT**  
**PILOT EVALUATION OF GPS TECHNOLOGY IN CHRONIC WASTING DISEASE**  
**PREVALENCE AND MANAGEMENT AT ARTIFICIAL FEEDING SITES IN URBAN AREAS**

Eric J. Bergman, Michael W. Miller and L. L. Wolfe

**INTRODUCTION**

Analyses of data from recent field studies and from culling have revealed areas of relatively high CWD prevalence associated with urban areas along the northern Front Range (Wolfe et al. 2002, 2004; Conner and Miller 2004; Farnsworth et al. 2004). Within these, artificial and illegal feeding sites may be particularly important because they appear to congregate deer in one location, thereby increasing local deer density and exposure to contaminated environments (Miller et al. 2004). Although the nature of the relationship between disease prevalence and mule deer density has not been definitively identified, it seems likely (Barlow 1996) that CWD prevalence is being indirectly elevated through artificial feeding. The development of global positioning system (GPS) technology and its incorporation into radio collars for wildlife research presents a tool for better understanding CWD in urban areas. We have initiated a pilot field study to: 1) evaluate the effectiveness of different GPS collars in identifying illegal feed sites in urban settings, and 2) develop and evaluate a strategy for utilizing GPS technology in studying and managing CWD in urban mule deer populations.

**METHODS**

The study area for this work is located within two subdivisions in Estes Park, Colorado. The subdivisions, separated by approximately 1.6 km, were identified as treatment and control sites based on the presence and absence of known feeding sites (Fig.1, Wolfe et al. 2004). Between five and eight adult (>1 yr old) female deer from each subdivision were captured and collared with one of two different brands of GPS collars (HABIT Research, British Columbia, Canada and LOTEK Wireless, Ontario, Canada). Collars from each company will be evenly distributed between sites. Capture will occur as part of an ongoing "test and cull" research project (Wolfe et al. 2004) during April 2004 and from August to October of 2004 as needed. Deer will be recaptured and collars will be removed prior to battery failure (~220 days service) in order to retrieve GPS data.

No specific hypotheses are being tested in this pilot study; rather, we are attempting to determine if GPS radio collar technology is adequate for use as a tool in refining CWD epidemiology and management. We will record and report on the performance of GPS collars, and calculate costs (mean, range per animal tested) associated with our artificial feed site identification strategy as implemented in this pilot study. However, we will compare home range sizes of deer from each site to determine if artificial feeding reduces home range size of deer. We will also incorporate ground survey data (Wolfe et al. 2004) to estimate and compare mule deer density and ultimately CWD prevalence from sampled deer at each site. CWD prevalence will be compared between sites as well as to previous estimates from the greater Estes Park area (Wolfe et al. 2004) to explore future research potential.

## RESULTS AND DISCUSSION

### GPS Collar Comparison

A total of 16 GPS collars (10 LOTEK, 6 HABIT) were available for testing in this study. Prior to initiation of this study no HABIT collars were on hand for deployment, rather, all 6 had to be built to specification and delivered. GPS collars from HABIT Research, ~\$1,800/unit, were programmed to: collect GPS locations every 2 hours, to transmit GPS data (via VHF signal) over two day intervals every two weeks and to transmit the most recent GPS location (via VHF signal) at the start of each minute. Due to delays in the manufacturing process, no HABIT collars were received in time for spring deployment ( $\geq 2$  weeks pre-fawning). Additionally, due to programming errors, 0 of 6 HABIT collars were ready for deployment after initial testing. Upon servicing by HABIT Research (~3.5 weeks), 3 of 6 collars appear to be ready for deployment in late summer 2004. The remaining HABIT collars (3 of 6) will be serviced and deployed upon satisfactory performance.

All LOTEK collars were on hand prior to initiation of this study. Eight of 10 collars were deployed in spring of 2004, with 1 of 10 needing service. GPS collars from LOTEK Wireless, ~\$3,500/unit, were also programmed to collect GPS locations every 2 hours, but did not offer remote download capabilities. All GPS locations collected by LOTEK collars will be acquired upon retrieval of the collar.

### GPS Collar Performance

Data from LOTEK GPS collars continues to be collected and HABIT GPS collars will be deployed between August-September 2004.

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- \_\_\_\_\_, M.W. Miller, and E.S. Williams. 2004. Feasibility of "test-and-cull" for managing chronic wasting disease in urban deer. *Wildlife Society Bulletin* 32:500-505.

Prepared by

\_\_\_\_\_  
Eric J. Bergman, Wildlife Researcher





Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. : Mammals Research  
Work Package No. 3740 : Mammals Support Services  
Task : Veterinary Services – Medical Support

Federal Aid Project N/A :

Period Covered: July 1 2003 through June 30, 2004

Author: L. L. Wolfe

Personnel: M. W. Miller, L. A. Baeten, M. M. Conner, K. Cramer, T. R. Davis, K. Griffin, D. O. Hunter,  
J. E. Jewell, E. Knox, C. E. Krumm, C. T. Larsen, J. Rhyan, M. Sirochman, T. Sirochman, E. S.  
Williams, D. Wroe

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**JOB PROGRESS REPORT  
VETERINARY SERVICES – MEDICAL SUPPORT**

L.L. Wolfe

**INTRODUCTION**

Veterinary services are provided as support for a variety of wildlife research projects, transplants and reintroductions conducted by the Colorado Division of Wildlife (CDOW) and its collaborators throughout the year. The following overviews and summarizes key wildlife veterinary medical support services provided during 2003–2004.

**VETERINARY MEDICAL SUPPORT**

Location of services & primary investigator	Species	Type of medical support
CDOW Foothills Wildlife Research Facility (FWRF), Tracy Davis and researchers	mule deer, white-tailed deer, elk, bighorn sheep, pronghorn, puma, others	Preventive, routine, and emergency medical care for all research animals housed at FWRF for use in ongoing CDOW and research.
Rocky Mountain Arsenal, Sherry Skipper	mule deer, white-tailed deer	Chemical immobilization of adult does for survival study and CWD surveillance. Does were ultrasounded, tonsil biopsied, blood was collected, and vaginal implant transmitters (VITs) were inserted.
Uncompahgre Plateau, Chad Bishop	mule deer	Medical care of injured animals, assisted with ultrasound, VIT, and blood collection for viral serosurvey and thyroid study.
Pinion Canyon maneuver site, Elizabeth Joyce	swift fox	Swift fox kits were anesthetized and abdominal radiotransmitters were surgically inserted and blood was collection.
Colorado Springs, Brian Dreher	mule deer	Adult deer were captured and radiocollared for CWD surveillance. We tonsil biopsied deer, collected blood, and provided area training for future efforts
CDOW FWRF, Department of Defense contract in cooperation with Elizabeth Williams	mule deer, white-tailed deer	Provided medical care for hand raised deer fawns, including diarrhea outbreak management and treatment of injured fawns.

**TRAINING**

Capture and Sampling A capture and handling training class was provided for the district wildlife manager trainees. A second class was held for researchers and biologists. Capture classes included lectures on drug use regulations and recordkeeping, pharmacology of select capture drugs, dosing, safety and types of equipment. These classes also included “hands on” capture and handling of animals at FWRF. This last year, we also devised and administered a written and practical exam for the DWM trainees. As needed, spaces in these classes also provide opportunities for graduate students and technicians to learn sample collection techniques.

## TONSIL BIOPSY

Tonsil biopsy training sessions were provided for staff from Wyoming Game and Fish Department and the Wisconsin Department of Natural Resources. In addition, CDOW personnel from the Colorado Springs area were trained on-site in capture and sampling techniques. Tonsil biopsy training sessions included lectures on sampling techniques and recognizing signs of CWD. The training also involves “hands on” time in the necropsy lab for sampling techniques. We also provide hands on training, as scheduling allows, on research animals.

## TARGETED CWD SURVEILLANCE

A training module was developed and used to instruct USFWS and tribal biologists in recognizing signs of CWD as a tool in developing targeted surveillance programs on national wildlife refuges and tribal lands. This training included a lecture and PowerPoint tutorial illustrating clinical signs of CWD, as well as first-hand observation of captive mule deer showing signs of CWD. The tutorial file was subsequently modified and made available to other state and federal agencies as a self-teaching tool to aid in respective CWD management programs.

## TRANSLOCATION

During transplant and translocation operations, we provide emergency medical care or humane euthanasia to injured animals. We also provide blood sampling, health exams, health certificates, vaccinations, anthelmintics, and antibiotics as needed to assure safe transport and improve survival in translocated wildlife.

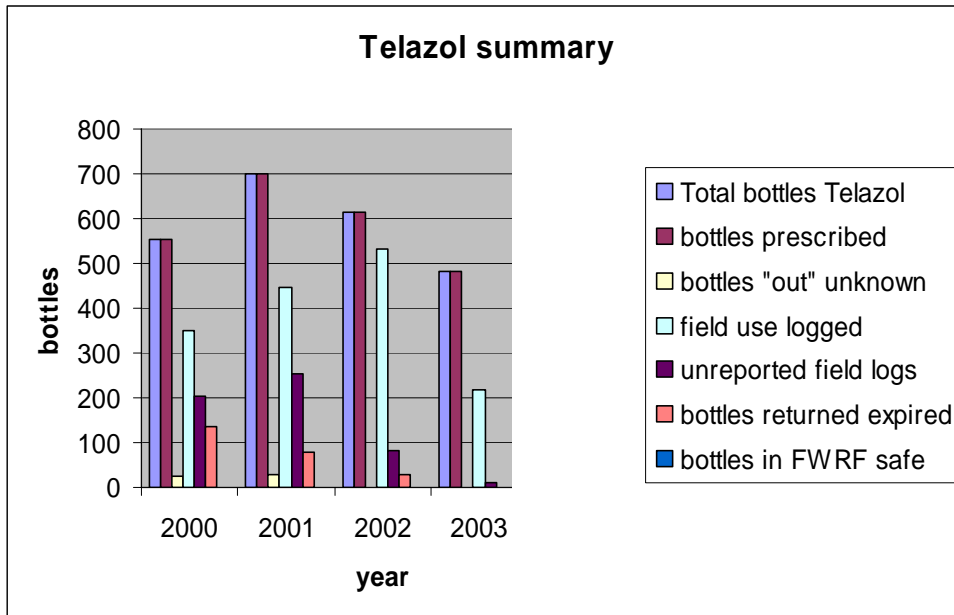
Species	Services Provided	Comments
BHS	Vaccination, anthelmintics, antibiotics, pharyngeal swabs	Transplanted within Colorado
swift fox	Health exams and health certificates	Released in South Dakota
black-footed ferrets	Health exams and health certificates	Released in Utah
Lynx	Entry and release exams, medical care for capture injuries	Reintroduction project*

**\*Lynx reintroduction:** Thirty eight lynx were received for the 2004 release. Most of the lynx were in good condition on arrival at the lynx holding facility in Del Norte. Three lynx required digit amputation due to trapping injuries, but all three healed without complications. One female was euthanized due to a compound fracture of the radius-ulna.

In 2003/2004, the anesthetic protocol for transported lynx was changed from 2.0-2.5 mg/kg Telazol<sup>®</sup> delivered intramuscularly (IM) to 20-40 mg (0.02 -0.05 mg/kg) ketamine and 0.6-0.8 mg (0.05-0.11 mg/kg) medetomidine given IM. No adverse anesthetic reactions were seen. Lynx were given the ketamine/medetomidine by IM injection while held in a squeeze cage. Induction time averaged 5.1 minutes (S.E. 0.4). Anesthetic time (induction to reversal) averaged 24.5 minutes (S.E. 0.9). Lynx were given atipamazole (0.25–0.55 mg/kg) in equal volume to medetomidine by IM injection. Lynx were recovered with minimal stimulation in their den boxes. Recovery time (time from reversal to standing with coordination) averaged 48.5 minutes (S.E. 2.5). There were no poor recoveries or anesthetic reactions. This new drug combination offered substantial reduction in processing and recovery times for lynx being handled at different points in the reintroduction process.

## DRUG DISTRIBUTION

Since 2002, there has been extensive reorganization of drug distribution procedures and recordkeeping for chemical capture of wildlife. Overall, there has been a dramatic improvement in drug tracking and accountability. This has resulted in a reduction in wasted expired drugs and improvement in field logs.



**CLINICAL TRIALS**

The following table summarizes the clinical trials from 2003. These trials were designed and conducted to improve veterinary medical care associated with various research and management programs conducted by CDOW. More complete reports of these trials are in the appendices.

Clinical Trial	Investigators	Species	Appendix
<i>Clostridium perfringens</i> type A vaccine trial	Wolfe, Miller, Davis, Ellis	BHS, MD	A
Plague vaccine in Canada lynx	Wolfe, Shenk, Baeten, Miller, Roke	lynx	B
Chemical immobilization field trial with medetomidine and ketamine combination	Wolfe, Miller	mountain lions, MD, WTD	C
Chemical immobilization with A-3080 in mule deer	Wolfe, Miller, Lance	MD	Published
Chemosterilization with GnRH toxin in mule deer	Baker, Wolfe,	MD	See progress report for Dan Baker
Comparison of dart injection quality between 2 brands of collared and uncollared darts in fallow deer	Wolfe, Ryan, Miller	fallow deer	D

## APPENDIX A

### EXPERIMENTAL EVALUATION OF A VACCINE FOR *CLOSTRIDIUM PERFRINGENS* TYPE A IN CAPTIVE BIGHORN SHEEP (*Ovis canadensis*) AND CAPTIVE MULE DEER (*Odocoileus hemionus*)

L. L. Wolfe, R. P. Ellis, K. Fox, T. Davis, and M. W. Miller

#### INTRODUCTION

*Clostridium perfringens* is found naturally in the intestines of animals and in the environment. This bacterium possesses the ability to produce heat-resistant endospores and potent extracellular toxins. Isolates of *C. perfringens* can be subdivided into types based on the production of these exotoxins. The four major toxins implicated in disease are  $\alpha$ ,  $\beta$ ,  $\epsilon$ , and  $\iota$ . Of these four major toxins, type A produces  $\alpha$  toxin only, type B produces  $\alpha$ ,  $\beta$ , and  $\epsilon$ , type C produces  $\alpha$  and  $\beta$ , type D produces  $\alpha$  and  $\epsilon$ , and type E produces  $\alpha$  and  $\iota$  toxins. Other minor toxins also exist within the five types of *C. perfringens*, although they are not used to identify the specific type due to overlap between types. These toxins include  $\delta$  (found in types B and C),  $\theta$  (found in all five types),  $\kappa$  (found in all five types),  $\lambda$  (found in types B, D, and E),  $\mu$  (found in types A, B, C, and D),  $\nu$  (found in all five types), and neuraminidase or sialidase (found in all five types). In addition, *C. perfringens* enterotoxin (CPE) is often produced. CPE is most often found occurring with type A, although it has also been documented with all five types of *C. perfringens*. Many toxins produced by *C. perfringens* organisms are hydrolytic enzymes, necessary for life as a saprobe found naturally in the soil. Type A, the focus of this study, also possesses enzymes with hydrolytic properties, including phospholipase C and sphingomyelinase activities (from the  $\alpha$  toxin). (Petit et al. 1999)

*Clostridium perfringens* type A has recently been implicated as a cause of enterotoxemia in a variety of species including lambs and goats. Tympany, hemorrhagic enteritis and abomasitis, and abomasal ulceration in calves characterize the disease. Lesions include necrotic enteritis in domestic chickens; necrotizing enterocolitis and villous atrophy in suckling and feeder pigs; and hemorrhagic gastroenteritis in dogs. (Bueschel et al. 1998). Other reports of enteric disease associated with *C. perfringens* type A include enterotoxemia in minks, muskrats, and racing camels, acute toxemia in water buffaloes (Songer, 1996), gastroenteritis in black-footed ferrets (Schulman et al., 1993) and dairy cattle (Dennison et al., 2002).

The  $\alpha$  toxin in type A *C. perfringens* acts by way of phospholipase C activity and sphingomyelinase activity, breaking down phosphatidylcholine and sphingomyelin found in the membranes of erythrocytes, platelets, leukocytes and endothelial and muscle cells. By way of this action,  $\alpha$  toxin is thought to be responsible for the cytotoxicity, necrosis, and hemolysis observed with type A *C. perfringens*. There is evidence suggesting that minor differences in the amino acid sequence of  $\alpha$  toxins exist, creating two strains with different pathways of infection. One strain has an increased resistance to chymotrypsin, allowing survival and multiplication in the gut, followed by entry into circulation. This strain is believed to be the primary cause of type A related enterotoxemia. The other strain, lacking a resistance to chymotrypsin, is believed to have a higher affinity for invasion of muscle tissue, and perhaps the cause of type A related gas gangrene (Songer, 1996).

Clinical signs of animals suffering from type A *C. perfringens* vary from species to species, but consistently include depression, anorexia, diarrhea, bloating in non-avian species, and death. Postmortem findings from these cases varies from species to species and between specific cases, but consistently tend to include gram positive bacilli surrounding necrotic tissue, necrosis, particularly in the small intestine,

and hemorrhage and ulceration, again in the small intestine; in ruminants, abomasitis, tympany, and abomasal hemorrhage and ulceration are also common findings.

Infection by type A *C. perfringens* is believed to occur in a variety of ways. One theory, especially in neonatal ruminant cases, is that engorgement on milk or esophageal groove dysfunction allows milk to spill over into the rumen, providing a substrate for growth of the bacterium, as well as an anaerobic environment in which to proliferate. Another suggested scenario, as found in cattle herds in Nebraska and Wyoming is that bacterial infection is secondary to copper deficiency. The findings of this study indicated that low copper concentrations may have weakened the abomasal mucosa and compromised immune function (Roeder et al., 1988). Environmental contamination may play a role in the acquisition of *C. perfringens* type A because these toxins are known to exist in the soil and many ruminant species ingest soil in attempts to acquire essential minerals. In addition,  $\alpha$  toxin can be detected in the feces of birds with necrotic enteritis (Bueschel et al., 1998), and thus avian vectors may provide an additional method of toxin movement.

The presence of *C. perfringens* type A at the Foothills Wildlife Research Facility (FWRF) in Ft. Collins, Colorado appears to be relatively recent, with the first case identified in 1997. Since then, the number of cases has increased exponentially, approximately doubling each year. A total of 29 cases had been attributed to *C. perfringens* type A since the first case was diagnosed in 1997. At the FWRF, the disease has affected primarily bighorn sheep and mule deer; these 2 species account for 25 of the 29 cases. In adult animals, bighorn sheep have been the primary species affected (6 out of 10 cases), and sudden death has been common. These animals often exhibited bloating and diarrhea shortly before death, and showed signs of hemorrhage such as bleeding from the mouth or anus. Necropsies of these animals consistently included large amounts of rod-shaped bacteria, especially in the small intestines. Other lesions included necrosis and hemorrhage, particularly in the heart and small intestine as well as intestinal and abomasal ulcers.

In neonatal and juvenile animals (<1 yr old), mule deer have been the primary species affected (14 of 19 cases), and chronic symptoms have been most common. These animals consistently exhibited chronic bloating, emaciation, depression, and soft brown diarrhea that was sometimes chronic, usually present early on in the animal's life, and often never alleviated despite various treatment attempts. Therapies included a barrage of antibiotics (benzathine penicillin and florfenicol appeared most effective), subcutaneous fluids, transfaunation, kaolin pectin with lactobacillus granules, probios powder, electrolytes, and medicated "Deccox" feed distributed by Ranchway Feeds. Despite therapy, most of these cases ended in death -- those that survived exhibited symptoms that were short-lived and often only exhibited a single case of bloating and/or diarrhea. Necropsies of affected animals consistently included: abomasitis; hemorrhage and ulcers in the intestine, abomasum, and lungs; fluid and gas throughout the intestines; watery to frothy green fluid in the rumen, and sometimes extending into other stomachs; necrosis; and rod-shaped bacteria in the abomasum and/or small intestine. Of the 19 neonatal cases, 5 occurred in bighorn sheep, and it is noteworthy that these cases were primarily in lambs born late in the spring, after the majority of lambing had already occurred. These cases were similar to those occurring in mule deer neonates.

Mortality caused by *C. perfringens* type A is a growing impact on FWRF operations and ongoing research: it is the leading cause of death in captive bighorns and second only to chronic wasting disease (CWD) as cause of death in mule deer. Moreover, because infections occur primarily in juvenile animals, many long-term studies (e.g., CWD and fertility control) have been hampered by lack of available animals for planned experiments. Here, we proposed to develop and evaluate efficacy of a vaccine to prevent *C. perfringens* type A morbidity and mortality in captive bighorn sheep and mule deer.

## METHODS

Initial vaccine development was pursued in Dr. Robert Ellis' laboratory in the Department of Microbiology, Immunology, and Pathology at Colorado State University.

We used captive Rocky Mountain bighorn sheep (*O. canadensis canadensis*) and mule deer (*Odocoileus hemionus*) in this experiment. All animals were housed at the CDOW's Foothills Wildlife Research Facility (FWRF) throughout the study and resided in 3-7 ha pastures. In addition to natural forage, grass/alfalfa hay mix and a pelleted high-energy supplement was provided as prescribed under FWRF feeding protocols for bighorn sheep and mule deer in respective age/sex classes throughout the study; fresh water and mineralized salt blocks was provided *ad libitum*.

The general health of all animals was evaluated immediately after vaccination, as well as daily thereafter, and observations recorded throughout our experiment. Injection sites were also examined weekly for 4 weeks after vaccine administration to assess local reactions to vaccine.

Bighorn sheep (n = 19) and Mule deer (n = 10) were randomly assigned to vaccinated or unvaccinated groups. The vaccinated group was injected IM with the *C. perfringens* vaccine in the right hind leg on day 0 and in the left hind leg 4 weeks later (booster). Blood was collected prevaccination, at the booster injection and 4 weeks after the final booster. The control group was weighed and blood was drawn at the time of the vaccine group's booster and 4 weeks after the final booster. Serum was separated and stored frozen until it was submitted to Colorado State Diagnostic Lab for antibody titer using enzyme-linked immunosorbent assay (ELISA).

As necessary deer were sedated with xylazine HCl (5-20 mg IV or 25-100 mg IM) or immobilized with a cocktail of thiafentanil HCl (8-10 mg), or ketamine HCl (100 mg), and xylazine HCl (20 mg), delivered IM by projectile syringe, to facilitate collections; narcotic effects were reversed with naltrexone HCl (150 mg SC + 50 mg IV).

## RESULTS

There were no vaccine site reactions or adverse effects from vaccination observed. No serum neutralizing antibody titers to *C. perfringens* were seen in either the BHS or MD. On follow up evaluation of the vaccine by Colorado State Diagnostic Lab, there was no type A antigen in the vaccine.

## DISCUSSION

The vaccine in this study failed due to lack of quality control by the manufacturer, however, we anticipate that a safe and effective vaccine can be readily developed, and that its incorporation into FWRF's preventive animal health program will reduce morbidity and mortality associated with *C. perfringens* type A infection. Managing clostridial enteritis is essential to improving success of preventative health programs at the FWRF and minimizing impacts on planned and ongoing research.

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## APPENDIX B

### SAFETY AND EFFICACY OF RECOMBINANT F1-V FUSION PROTEIN VACCINE TO PROTECT LYNX FROM PLAGUE

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#### INTRODUCTION

Plague, caused by *Yersinia pestis*, was introduced into the North American continent in the early 1900s, and its impacts on some native wildlife species since that time have been substantial (Cully 1993, Wuerthner 1997, Gasper and Watson 2001). Epidemics in prairie ecosystems have been well documented, and probably contributed to the marked declines observed in both prairie dogs (*Cynomys* spp.) and black-footed ferrets (*Mustela nigripes*) over the last century (Cully 1993). Although less extensively studied, it seems likely that sylvatic plague has impacted other wildlife species as well (Gasper and Watson 2001).

Canada lynx (*Lynx lynx*) resided in Colorado historically (Fitzgerald et al. 1994), but apparently were extirpated by the late 1970s. Whether plague played any role in the disappearance of lynx from Colorado is not known. Regardless of plague's role in the historical decline, this disease now appears to be an obstacle to ongoing efforts to reestablish lynx in southwestern Colorado. To date, *Y. pestis* infections have been confirmed in 6 Colorado lynx. Plague was the primary cause of death in 27% (4/15) of the known natural deaths and possibly contributed to 1 of the 6 known hit-by-vehicle deaths in adult lynx released in Colorado since 1999 (Wild 2000, Shenk 2003; T. M. Shenk, Colorado Division of Wildlife, unpublished data). Plague also killed at least 1 kitten born in the wild during the first year of documented natural reproduction in Colorado's reintroduced lynx population (T. M. Shenk, Colorado Division of Wildlife, unpublished data). Practical tools for preventing plague in reintroduced lynx could benefit species recovery efforts in Colorado and perhaps elsewhere.

Effective vaccines for preventing plague in mammalian species, including felids, have been developed only recently (Heath et al. 1998, Gasper and Watson 2001, Creekmore et al. 2002). Of these, a recombinant capsular F1-V fusion protein vaccine (Heath et al. 1998) has shown a promising combination of safety and efficacy in black-footed ferrets (Rocke et al. in press), and could be useful in lynx restoration as well. Here, we propose to (1) evaluate F1-V vaccine in captive lynx being held in southwestern Colorado prior to release as part of an ongoing restoration program and (2) compare number of lynx mortalities caused or complicated by plague in vaccinated and unvaccinated lynx released in Colorado.

#### METHODS

Our study was conducted in conjunction with the 2004 release program. All lynx were captured, transported, held, cared for, and handled as described in established protocols for Colorado's restoration program (Wild 2000). Lynx were held at the Frisco Creek Wildlife Rehabilitation Center (FCWRC) prior to and throughout the study until release. Whenever possible, vaccination and sampling was done in

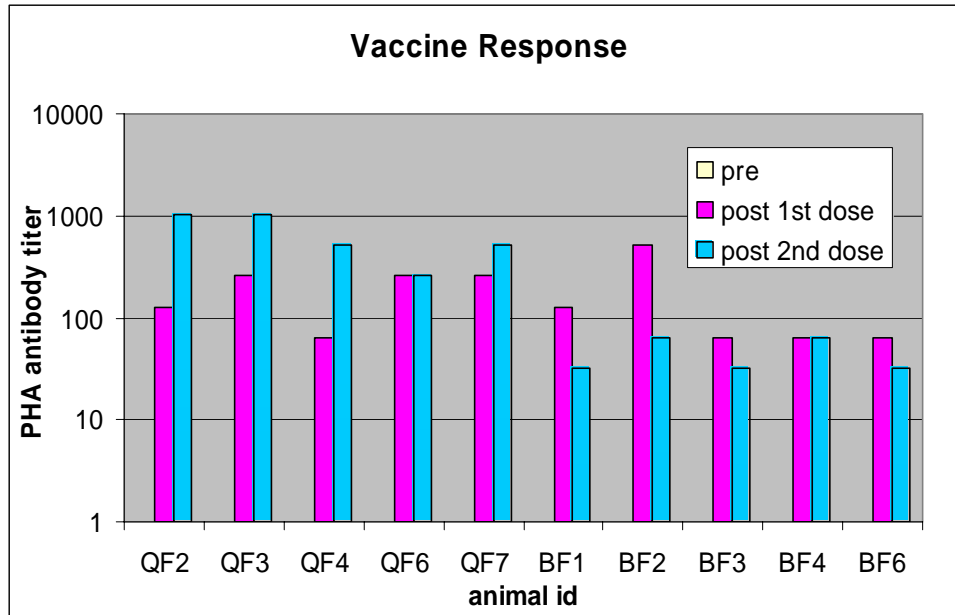
conjunction with other handling activities to minimize stress that could arise from repeated handling of captive lynx.

We initially evaluated safety and efficacy of F1-V vaccine (U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD) in 10 adult lynx; 10 age- and origin-matched lynx will remain unvaccinated as controls. Blocks will consist of age and origin: age will be either  $\leq 5$  years old or  $\geq 6$  years old; origin will be either British Columbia (where prior exposure to plague is possible) or Manitoba/Quebec (where prior exposure is unlikely). We estimated ages based on tooth wear; animals  $\leq 1$  year old were excluded. Within each block (age and origin) of lynx, half were selected at random to receive the vaccine while the remainder will serve as controls. Vaccine was diluted and combined with Alhydrogel adjuvant (United Vaccines, Madison, WI) as described by Rocke et al. (in press). We administered vaccine via subcutaneous (SQ) injection in the hindquarter on day 0 and a second dose was given 21 days later. Initial vaccine doses were delivered by hand-held syringe when lynx are examined upon entry into FCWRC; booster doses were delivered via hand-held syringe while the lynx was restrained in a squeeze cage.

Vaccinated lynx were observed immediately after vaccination, immediately upon recovery from anesthesia (when applicable), and daily thereafter for evidence of adverse effects. To evaluate serological responses of vaccinated lynx as an index of efficacy, we will collect blood (~6 ml) from all captive lynx at each handling during the 2004 season regardless of vaccination status. For the 10 principal vaccinates and controls, at minimum blood will be collected on day 0 and again 42 days later (21 days after the booster vaccination). Serum was harvested and stored frozen until assayed. We will measure antibody titers against F1 and V antigens with phytohemagglutination assay (PHA) at the Center for Disease Control and an enzyme-linked immunosorbent assay (ELISA) using methods of Rocke et al. (in press). For the 10 principal vaccinates and controls, we compared changes in  $\log_{10}$  anti-F1 and anti-V antibody titers<sup>-1</sup>. Mortality of vaccinated lynx due to or complicated by plague will be compared to mortality due to or complicated by plague of unvaccinated lynx from previous releases. A suite of models developed *a priori* will be evaluated through AICc model selection (Burnham and Anderson 2002) to investigate the possible effects of vaccination status, age, location of birth, and time to death on mortality of lynx due to or complicated by plague.

## RESULTS

All PHA prevaccination titers were negative. All vaccinated lynx showed seroconversion on the PHA assay at the after the first and second booster (figure 1.). ELISA results are pending. There were no vaccine site reactions and no adverse side effects were seen.



**Figure 1.** PHA antibody titer for individual lynx. All pre titers were negative. All lynx showed seroconversion following vaccination.

## DISCUSSION

Lynx were examined on entry and 5 females from Quebec and 5 females from British Columbia were randomly chosen for vaccination with F1-V fusion protein plague vaccine. All pre vaccine PHA titers were negative. All vaccinates showed seroconversion but the quantitative titer assays are still pending. The vaccine appears to be safe in lynx; there were no vaccine site reactions or adverse systemic reactions.

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## APPENDIX C

### **EFFICACY OF KETAMINE MEDETOMIDINE COMBINATION IN MOUNTAIN LIONS (*Puma concolor*), MULE DEER (*Odocoileus hemionus*) AND WHITE-TAILED DEER (*Odocoileus virginianus*) FOR CHEMICAL IMMOBILIZATION IN THE FIELD**

L. L. Wolfe, W. R. Lance and M. W. Miller

In cooperation with Wildlife Pharmaceuticals, Inc. (Fort Collins, CO) we are using ketamine (200mg/ml) and medetomidine (20 mg/ml) compounded at a higher concentration than commercially available. By concentrating the drugs we are able to use an effective dose in a 1 cc dart for mountain lions and a 2 cc dart for deer. To date over 200 deer have been captured and 5 mountain lions using this combination. No adverse side effects have been seen. Evaluation of this drug combination for field capture is ongoing.

## APPENDIX D

### EVALUATION OF COLLARED AND UNCOLLARED DANINJECT AND PNEUDARTS

L. L. Wolfe, D. M. Okeson, W. R. Lance, J. Rhyan, and M. W. Miller

#### INTRODUCTION

Remote delivery systems, powered by compressed CO<sub>2</sub> or blank charge, are an important tool for wildlife immobilization. Darts used with these remote delivery systems are barbed, collared or have uncollared needles. The purpose of the barbs and collars are to hold darts in place long enough to ensure complete drug delivery. However, barbed darts can only be used to deliver anesthetics, thus allowing for dart retrieval. Collared darts and uncollared darts will fall out on their own, but drug delivery may be incomplete. Drugs are delivered from the darts with a powder charge (Pneu-dart™, Williamsport, PA) or pressurized air (Daninject™, Wildlife Pharmaceuticals, Fort Collins, CO). Some level of trauma is inherent, and varies greatly with the type of dart used (Valenburg et al. 1999, Kreeger 2002). Powder charged darts deliver drug rapidly, but are potentially more traumatic than the air pressurized darts; consequently, induction times vary when these darts are used to deliver anesthetic drugs.

In this study we compared drug delivery between collared and uncollared darts. We compared dart trauma between collared and uncollared darts and we compared Daninject darts with Pneu-darts.

#### METHODS

This study was conducted in conjunction with a previously-approved terminal study testing fallow deer susceptibility to chronic wasting disease (CWD) (CDOW ACUC 12-2000). Because these animals were already slated for euthanasia, we opportunistically evaluate and compare trauma and drug delivery associated with the respective dart types immediately prior to euthanasia.

Deeply anesthetized fallow deer were placed on a stand to facilitate darting the hindquarters. Each animal was darted in the hindquarter with a collared Daninject dart and Pneu-dart dart on one side and uncollared Daninject dart and Pneu-dart dart on the opposite side. Darts were loaded with 2 cc India ink. The animals were then be euthanized by intravenous injection of Euthansol (8.8 mg/kg). Each dart site was evaluated for amount of India ink leakage at dart site, degree of trauma (recorded on a scale of 0-3. (0= none, 3 = extensive hemorrhage and tissue disruption) and ink injection pattern.

All fallow deer were be captured with thiafentanil oxalate (0.1 mg/kg) delivered intramuscularly (IM) via projectile syringe using an adjustable air-powered rifle and xylazine hydrochloride. Anesthetic drugs were delivered to the shoulder and neck to avoid confounding subsequent assessment of dart effects.

#### RESULTS

On necropsy the ink pattern at the injection site was evaluated. A common ink pattern noted at necropsy was a “T” shaped pattern. The superficial area of ink (top of T) averaged 20-25 mm in diameter (recorded on line #4- spread of ink in muscle). Note that this refers only to the most superficial spread (horizontally) of the ink in the muscle; ink forming the top part of the T was typically only 2 mm thick. Ink then typically followed the needle track 20-30 mm into muscle. The pattern of “focal” was often recorded, but would probably be more correctly stated as “along needle track” as these 20-30 mm deep tracks were typically only 2 mm wide (roughly the diameter of a needle).

Most injection sites had minimal hemorrhage or trauma this was recorded on a scale of 0-3. (0= none, 3 = extensive hemorrhage and tissue disruption) Overall, most dart sites were rated a “0” (12 of 32 darts) or a “1” (7 of 32 darts).

Uncollared PneuDarts

Eight of 8 uncollared PneuDarts bounced off the animal immediately after impact. However, ink was delivered into the animals, in all but one case. In 5 cases darts bounced, but no ink was noted on the hair of the animal or was observed spraying from the bounced dart. At necropsy, ink was noted in muscle in all of these cases, indicating that the bounced darts did deliver ink prior to ejecting from animal. One dart that bounced was noted to have sprayed a large amount of ink. However on necropsy, ink was found in muscle, indicating that dart did deliver at least some of the ink into the muscle. One dart bounced was noted as “did inject”, but some ink was noted on hair around injection site. At necropsy, ink was found in muscle, indicating that dart did deliver at least some of the ink into the muscle. One dart bounced was noted as “did inject”, but there was ink running down from the injection site. At necropsy, it was hard to distinguish any ink from bruising, so this dart may have not injected any ink into the muscle.

There were 2 of 8 animals scored as “3” (extensive hemorrhage and tissue disruption). However there were also 3 of 8 animals scored as a “0” (no hemorrhage and tissue disruption.). In addition 2 of 8 were NR (not recorded). It is difficult to draw a conclusion as to whether or not there is a tendency for this type of dart to cause more tissue damage.

Fallow deer dart study 4/16/03 - Results for smooth Pseudart (symbol +)							
Deer #	Dart stayed in muscle?	Ink on hair	Depth of ink in muscle	Pattern of ink in muscle	Superficial spread of ink on muscle	Hemorrhage/trauma	Fits typical T pattern of ink spread?
1102	N	1	5 mm	NR	25 mm	NR	?
202	N	NR	Unable to distinguish ink from hemorrhage/bruising. Did dart inject?			2	unknown
302	N	3	20 mm	deep, focal	20 mm	0	?
2002	N	1	located 20 mm deep	15 mm focal spot located 20 mm deep		3	N
1002	N	1	5 mm	dissecting on tendon sheaths	5 mm	0	N
1802	N	0	spot located 20 mm deep	focal, but no needle track	60 mm superficial spot plus spot located 20 mm deep into muscle with a 20 mm diameter	3	Y
2502	N	0	< 2 mm	only superficially delivered		0	N
1202	N	1	20 mm	NR	20 mm	NR	?

Collared PneuDarts

All 8 collared PneuDarts stayed in the animal after impact. In 3 of 8 cases the darts delivered ink only very superficially (not deep in muscle).

Overall the collared darts caused very little trauma. There were 2 of 8 animals with a rating of “0”, and 2 of 8 with a rating of “1”. Only 1 of 8 animals had a rating of either “2” or “3”. Not recorded = 2 of 8.

Fallow deer dart study 4/16/03 - Results for collared Pseudarts (symbol #)							
Deer #	Dart stayed in muscle?	Ink on hair	Depth of ink in muscle	Pattern of ink in muscle	Superficial spread of ink on muscle	Hemorrhage/trauma	Fits typical T pattern of ink spread?
1102	Y	0	"very superficial" (?<2mm)	only superficially delivered	20 mm	2	N; Ink only superficially delivered, minimal muscle penetration of ink.
202	Y	0	20 mm	deep, focal	5 mm	3	Not sure of pattern
302	Y	0	< 2mm	70 mm superficial "splotch", dissects along fascial planes	70 mm	0	N; Ink only superficially delivered with spread along superficial fascial planes
2002	Y	0	20 mm	focal	20 mm	1	Y
1002	Y	0	30 mm	focal	5 mm	NR	Y
1802	Y	0	35 mm	focal	5 mm	1	Y
2502	Y	0	< 2mm	only superficially delivered	60 mm	0	N; Ink only superficially delivered, minimal muscle penetration of ink.
1202	Y	0	15 mm	NR	20 mm	NR	?

### Uncollared Dan-Inject Darts

Seven of 8 **uncollared** Dan-Inject darts stayed in the animal's muscle after impact. The result of one uncollared Dan-Inject dart was not recorded. Overall, the uncollared Dan-inject darts caused very little hemorrhage or trauma. Four of 8 animals had a rating of "0" hemorrhage/trauma rating and 2 scored 1 and 1 scored 2.

Fallow deer dart study 4/16/03 - Results for smooth DanInject darts (symbol *)							
Deer #	Dart stayed in muscle?	Ink on hair	Depth of ink in muscle	Pattern of ink in muscle	Superficial spread of ink on muscle	Hemorrhage/trauma	Fits typical T pattern of ink spread?
1102	Y	0	< 2mm	very superficial	15 mm	1	2 smooth Daninjects hit animal. 1st "penetrated leg, injection out back side of leg". 2nd dart also recorded as Yes stayed in muscle & 0 ink on hair; but not sure if depth, pattern, and spread info is for 1st or 2nd dart. ???
202	Y	0	0	no ink injected into muscle	0	0	N; dart went deep into limb but injected ink out medial aspect
302	Y	0	30 mm	deep, focal	20 mm	1	Y
2002	Y	0	50 mm	deep, focal	5 mm	0	Y
1002	NR	0	15 mm	focal	5 mm	0	Y
1802	Y	0	50 mm	NR (not recorded)	20 mm	0	Y?
2502	Y	3	75 mm	NR	10 mm	2	1st smooth Daninject dart went through leg, injected some out caudal aspect (3 for ink on hair refers to ink on back of limb from 1st dart). 2nd dart recorded as - stayed in muscle, no ink on hair; but not sure if 75 mm & 10 mm is for 2nd dart or 1st dart???
1202	Y	0	0	only superficially delivered	20 mm	NR	N; Ink only superficially delivered, no muscle penetration of ink.

### Collared Dan-Inject Darts

Seven of 8 collared Dan-Inject darts stayed in the animal's muscle after impact. The result of one collared Dan-Inject dart was not recorded.

These darts shows a tendency to cause little to no tissue damage. There were 6 of 8 animals with a rating of either "0" or "1".

These darts show a tendency to cause little to no tissue damage. There were 3 of 8 animals with a rating of "0", and 3 of 8 with a rating of "1". Only 1 of 8 animals had a rating of "3".

Fallow deer dart study 4/16/03 - Results for DanInject collared darts (symbol @)							
Deer #	Dart stayed in muscle?	Ink on hair	Depth of ink in muscle	Pattern of ink in muscle	Superficial spread of ink on muscle	Hemorrhage/trauma	Fits typical T pattern of ink spread?
1102	Y	0	5 mm	focal	15 mm	1	Y
202	Y	0	2 mm	deep, focal	10 mm	0	N, basically round superficial, spot
302	Y	0	20 mm	diffuse ~15 mm	10 mm "deep" (?)	1	? Not sure of pattern
2002	Y	0	deep 15 mm	dissects btw muscle masses	5 mm	3	? Not sure of pattern
1002	NR	1	25 mm	deep, focal	25 mm	1	? Not sure of pattern
1802	Y	0	40 mm	deep, focal	5 mm	0	Y? may be 5 mm wide all the way down
2502	Y	0	10 mm	deep, focal	25 mm	0	Y
1202	Y	0	35 mm	NR (= not recorded)	35 mm	NR	?; Note 0.5 mL of ink left in dart

## DISCUSSION

All of the collared darts stayed in the muscle (did not bounce). All of the Daninject uncollared darts also stayed in the muscle. Only the uncollared pseudarts bounced out of the muscle.

There was no inject sprayed on the hair of animals darted with collared pseudarts. Only one animal in each group of animals darted with daninjects had ink sprayed on the hair. Five of the animals darted with uncollared pseudarts had ink sprayed on the hair.

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Prepared by \_\_\_\_\_  
 Lisa L. Wolfe, Veterinarian



### JOB PROGRESS REPORT

State of <u>Colorado</u>	:	<u>Cost Center 3430</u>
Project No. _____	:	<u>Mammals Research</u>
Work Package No. <u>3740</u>	:	<u>Mammals Support Services</u>
Task No. <u>3</u>	:	<u>Animal and Pen Support Facilities for Mammals Research</u>

Period Covered: July 1, 2003 – June 30, 2004.

Author: T.R. Davis

Personnel: M. Anderson, K. Beamer, T. Bogardus, E. Crawford, E. Donegan, M. Dupire, K. Fagerstone, J. Faue, E. Featherman, K. Fox, T. Halasinski, L. Ho, M. Hanusack, G. Harvey, E. Jones, K. Kanapeckas, J. Kint, G. Kyriacou, I. Levan, T. McCollum, A. Mitchell, A. Northrup, M. Paulek, A. Phillips, R. Rhyan, T. Sanders, J. Sirochman, T. Sirochman, J. Stout, T. Stout, D. Thompson, R. Thompson, D. Weaver, A. Wilson

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### ABSTRACT

The Colorado Division of Wildlife's Foothills Wildlife Research Facility (FWRF) maintained captive animals (2003/2004 annual total: 360) and facilities in support of seventeen captive wildlife research projects. The primary focus of research during this period was chronic wasting disease (CWD) pathology, epidemiology, preventative therapies, sources of transmission in deer and potential transmission to other species. FWRF supported a number of other significant research projects including contraception and reproductive effects, pathogen immunization, evaluation of wildlife capture pharmaceuticals and personnel training in ante mortem sampling and field immobilization. The quality of animal care and facility maintenance provided by temporary, work-study, personal service, intern and volunteer employees is in part reflected by the finding of compliance under the Animal Welfare Act during the annual USDA inspection of FWRF. Herd management practices allowed pronghorn antelope and bighorn sheep herd levels to decline through natural mortality and the remaining domestic ferrets were removed as per study protocols. Alternatively, herd levels of mule deer and white-tailed deer were managed for maximum growth to support on going CWD research. Chronic wasting disease was again a significant source of mortality in mule deer and white-tailed deer and is reflected by the number of CWD research projects conducted during this period. We continue to manage CWD with the philosophy of managing the disease for research purposes under heightened bio-safety guidelines and intensive herd management. Fawn mortalities were higher than expected during the 2004 rearing season however a number of disease causing agents and contributing factors were identified through various diagnostic tests and evaluations. Neonate training was intensified for hand raised animals and training SOP's developed to accommodate CWD epidemiology research. Administrative actions include compiling a summary of all current and historic FWRF published research, an animal husbandry change order request was

implemented, and the FWRF tour policy revised. New SOP's were implemented for routine equipment maintenance, seasonal winterizing, tree/shrub care, and the construction maintenance work request forms were revised and reinstated. In addition to routine maintenance, the FWRF team made significant facility improvements including new facilities to accommodate CWD epidemiology research, completion of the mountain lion holding facility, and installation of a perimeter fence around the Wildlife Health Lab. The capitol construction team allocated funds for a new hay barn which is scheduled for replacement in the summer of 2005, and engineering assisted in the development of electronic facility site maps. In addition, the FWRF landowner; Colorado State University approved an easement for the Northern Colorado Water Conservation District to install a 68 inch water pipeline through the center of the facility (north to south). The installation process partially disrupted FWRF management activities for a six week period, but resulted in an upgraded road system, and replacement of existing windrows with five gallon potted trees and shrubs.

**JOB PROGRESS REPORT  
ANIMAL AND PEN SUPPORT FACILITIES FOR MAMMALS RESEARCH**

T.R. Davis

Animal Maintenance:

Routine animal husbandry including feeding, health observations, training, weighing, and clean-up, was performed primarily by well trained temporary employees, work-study students, and volunteers. FWRP was inspected by USDA APHIS for compliance with federal animal welfare regulations on July 28, 2004. Table 1 summarizes the number of animals by species reported to USDA animal welfare for the period of October 1, 2003 – September 30, 2004.

Table 1. Total number of animals by species reported to USDA Animal Welfare

<b>Species</b>	<b>Number of animals held, not dedicated to research</b>	<b>Number of animals dedicated to research</b>	<b>2003/2004 Total</b>
Bighorn Sheep	11	16	27
Elk	16	15	31
Fallow Deer	0	28	28
Mule Deer	75	84	159
Pronghorn Antelope	19	0	19
Sika Deer	1	0	1
White-tailed Deer	45	25	70
Cattle	0	11	11
<b>Ungulate Total</b>	167	179	346
<b>Ungulate Mean</b>			201
Domestic Ferrets	0	10	10
Prairie Dog	1	0	1
Mountain Lions	0	3	3
<b>Facility Total</b>	168	192	360

The number of animals held but not dedicated to research includes all animals being bred, conditioned, or held for use in research, but not yet used for such purposes. This group consists primarily of breeding animals, and young animals. The relatively high number of ungulates not dedicated to research during this period is the result of a large influx of young animals (born at FWRF, and orphaned neonates) dedicated to the CWD epidemiology study scheduled to begin data collections in the fall of 2004.

The total number of animals dedicated to research includes all animals used in experiments at any time during the period. Experiments include those involving no pain, distress, or use of pain relieving drugs, and experiments where pain relieving drugs were necessary to minimize stress on the animal. No animals at FWRF were used in experiments involving pain, without the use of anesthetic, analgesic or tranquilizing drugs.

The species total includes all adult animals housed at the facility, neonates born at the facility, transfers into and out of the facility, and all animals that died or were humanely euthanized during the respective fiscal year. It is important to note that ungulate herd levels at any one time averaged approximately 60 percent of the ungulate total and 55% percent of the total number of animals housed at the facility for the entire period.

#### Herd Management:

One habituated sika deer and one habituated prairie dog were brought into the facility to support division law enforcement efforts. Mule deer, white-tailed deer, and elk herd levels were expanded through herd management practices and incoming transfers to support CWD and fertility control research. Incoming transfers consisted primarily of habituated adult animals and orphaned neonates obtained from various locations around the state, as well as Wyoming, Nebraska, Iowa, and Kansas. The bighorn sheep and pronghorn antelope herds were reduced through natural mortality and out going transfers as the experiments these animals were dedicated to, reached a stage of completion. Eight fallow deer and the remaining domestic ferrets were removed through planned euthanasia as per the study protocols, while mountain lion and cattle numbers remained constant for the period.

Commission approval was granted in 2001 to transfer excess FWRF captive wildlife, and/or orphaned neonates out of state to support collaborative and non-agency wildlife research projects. In 2004 eight pronghorn antelope neonates were transferred to the National Wildlife Research Center (NWRC) in Fort Collins. Two of these animals were orphaned neonates, and six were excess animals of FWRF origin. Other facility transfers include a pronghorn buck that was borrowed from, and returned to, the Sybille Wildlife Research Unit in Wyoming.

FWRF herd management practices include planned breeding to maintain optimal population sizes of the various species required to support current and future research projects. Depending on research objectives, some of the offspring from FWRF animals are hand-raised, and various species of wild orphaned neonates are accepted for hand rearing. Habituated weanlings and adult animals are also accepted whenever herd levels will allow. Hand rearing protocols for mule deer are described by Parker and Wong (1987), and by Wild and Miller (1991) for bighorn sheep, elk, pronghorn antelope, and white-tailed deer. Table 3 summarizes the breeding and rearing practices of ungulate species for the period:

Table 3. FWRF Ungulate breeding and rearing practices

Species 2003/2004	FWRF Breeding 2003	FWRF Neonate Rearing 2004	Orphan/Transferred Neonates 2004
Bighorn Sheep	Bred 7 Ewes	Hand raised 2 Dam raised 3	0
Elk	Bred 3 Cows	Dam raised 3	1
Mule Deer	Bred 17 Does	Hand raised 16 Dam raised 11	32
Pronghorn Antelope	Bred 4 does	Transferred to NWRC 6	0
White-tailed Deer	Bred 9 does	Hand raised 6 Dam raised 7	24

Table 3 does not include three orphan mule deer fawns euthanized on arrival due to severe injuries, or very poor body condition, and five animals (3 mule deer, 2 white-tailed deer) which were still born or died shortly after birth due to parturition complications. The mountain lions, domestic ferrets, and fallow deer are also not included in the above table, as the mountain lions and domestic ferrets were neutered at an early age and the male fallow deer were vasectomies prior to the 2003 breeding season and therefore no breeding occurred in these species.

Nutritional Maintenance:

Feeding protocols for ungulates previously housed at the facility were reviewed by Wild (1997), and feeding protocols for the fallow deer and mountain lions were described by Davis (2003). The sika deer was maintained on a high quality grass alfalfa mix hay and Regular Ranch-way deer and elk ration. The prairie dog was maintained on Mazuri ADF # 25 herbivore diet, grass/alfalfa mix hay, and fresh vegetables.

Individuals of all species maintained reasonable body condition on available diets with the exception of some hand raised neonates (primarily mule deer fawns), and CWD infected animals at the clinical stage of the disease. Fawn mortalities may have been associated with general poor body condition of does infected with chronic wasting disease, the presence of other etiological agents identified (see health maintenance below), and/or interspecies competition for space and cover in paddocks housing cattle and fallow deer.

Pen Enrichment:

In an effort to provide cover and subsequently reduce stress, additional artificial refuge areas were constructed in paddocks housing semi-wild deer and dam raised neonates. "Y" shaped hide-outs, were constructed on site, vegetation ex-closures were added in early spring and removed later to enhance natural cover, and creep areas with natural cover were provided for dam raised fawns. All pen structure enrichments were readily accepted and utilized by the animals.

In addition to pen structure, behavioral enrichment was offered through training. Expanding on the operant conditioning system for mountain lions described by Davis (2003) hand raised ungulate neonates were "treat" trained using the same philosophy. Bighorn sheep, mule deer and white-tailed deer were taught to follow their human trainers and stand on the scale for physical exams, injections, treatments and weighing. Additionally, mule deer and white-tailed deer were gradually conditioned to the metabolic cages in preparation for CWD epidemiology sample collections. Passive training was used in conjunction with the above techniques to habituate animals to the scale and alley-way through supplemental feeding to encourage free exploration without human interference, in these areas.

### Health Maintenance:

Animal health care was provided as required and as mandated by the preventive medicine program (Wild 1995) and chronic wasting disease protocols. Overall, captive wildlife maintained at FWRF remained healthy throughout the period. Chronic wasting disease (CWD) continues to be a significant source of mortality in captive mule deer and white-tailed deer and is reflected by the number of animals dedicated to CWD research projects throughout this period. Mortality of an adult pronghorn doe was attributed to dystocia, and as described in previous years (Davis 2003) was associated with a failure of the cervix to dilate at the time of parturition. Several cases of dystocia with variable presentations were also observed in mule deer (n=2) and white-tailed deer (n=1). Epizootic hemorrhagic disease (EHD) and bluetongue virus (BTV) were not significant etiological agents during this period and may be associated with a management effort to reduce the quantity of free standing water on the facility, coinciding with the time of documented seasonal peaks of the disease.

Mortality rates and disease were higher than expected in hand raised mule deer fawns. Hand raised fawn mortalities were primarily associated with two types of illness: 1.) Intestinal disease resulting in diarrhea, bloating, and/or dehydration accompanied by a general lack of appetite and failure to thrive, and 2.) Respiratory disease (acute bacterial pneumonia) resulting in nasal discharge, coughing, labored breathing, and in some cases, no preliminary signs and acute death. Post mortem sampling and fecal isolation, revealed clostridium perfringens, salmonella, Escherichia coli, and rotovirus. Nasal cultures and post mortem sampling of lung tissue revealed mixed bacterial infections including, Alcaligenes species, Pasteurella species, Pseudomonas aeruginosa, however Arcanobacterium pyogenes was consistently diagnosed and is likely responsible for those cases resulting in acute death.

In addition to the etiological agents identified, several management and natural conditions may have contributed to fawn mortalities: 1) Inadequate hospital facilities, and clean isolation areas to separate sick animals (facility carrying capacity), 2) Higher than normal precipitation levels contributing to viable pathogens surviving in the soil for longer periods, and greater exposure to damp/cool conditions, 3) immuno-compromised animals to start with, as FWRF born fawns are exposed to a very pathogen rich environment at birth, and, a high percentage of the hand raised animals were orphans who are often in poor body condition and/or ill when they arrive. Due to animal welfare concerns, management recommends construction of adequate animal holding and hospital facilities prior to hand raising mule deer in the future, as well as a review of the neonate nutrition, health maintenance, and fawn rearing programs.

### Chronic Wasting Disease:

Following the recent revision of the CWD protocol (Davis 2003), we continue to manage CWD with the philosophy of managing the disease for research purposes under heightened bio-safety guidelines and intensive herd management. Intensive herd management is accomplished using the early detection techniques described by Wild et. al (2002) and Wolfe et. al (2002). All animals at FWRF were monitored closely for clinical signs of CWD, and tissues from all mortalities occurring at FWRF were examined for evidence of infection with CWD.

### Systems Development:

Administrative actions include compiling a summary of published articles generated from FWRF research. Hard copies of the 100 + articles filed by date of publication are available at FWRF and the research library. Currently, we are compiling an Access database of the articles to facilitate searches by author, subject, date, etc. In addition, an animal husbandry change order request was implemented as suggested by the Mammals research leader. The change order, modeled after the construction/maintenance work request, was designed to track the origin and justification of facility changes in herd stocking levels, species needs, and basic husbandry techniques including animal care, breeding, rearing, and training practices.

Other administrative actions include the development of new standard operating procedures for routine equipment maintenance, seasonal winterizing, and tree/shrub care. The SOP's are designed to put all FWRF equipment on a routine maintenance and winterizing schedule, and the schedule is specific to what level of maintenance is necessary at each interval. In the same fashion an SOP was developed for soil moisture testing and watering of tree and shrub windrows. Due to the increasing demands for unscheduled (but necessary and often emergency) construction and maintenance needs, the work request forms were revised and reinstated. The forms were designed to assist in prioritizing and assigning tasks, as well as provide a format for information transfer (an accurate description of the need), and to track labor costs associated with specific projects, routine and emergency maintenance.

#### Educational Contributions:

The FWRF tour policy was also revised. The revised policy allows for use of FWRF animals and facilities for hands on training of CDOW employees, collaborators, and other professional groups in sampling techniques and chemical immobilization when pre-approved by the Mammals research leader and/or the Animal Care and Use Committee (ACUC). FWRF functions primarily to support wildlife research, but will no longer function secondarily as an educational facility due to the overwhelming demand for this service. Protecting the integrity of the research, facility management, and increasing animal welfare concerns were sufficient justifications for the policy change.

#### Research Projects:

Facility operations offered support for research projects conducted by CDOW personnel and other collaborators that were initiated, conducted, or continued using FWRF animals and facilities. A total of twenty one research projects were supported by FWRF for the period:

- Cattle susceptibility to chronic wasting disease.
- Susceptibility of fallow deer to chronic wasting disease.
- Susceptibility of Mountain Lions to chronic wasting disease.
- Mechanisms of CWD transmission in mule deer.
- Evaluation of prospective preventative therapies for chronic wasting disease in mule deer.
- Validation of a potential blood test for chronic wasting disease (GeneThera test).
- Molecular epidemiology of strain variations in chronic wasting disease.
- Pathogenesis of chronic wasting disease in white-tailed deer.
- Effect of copper pathogenesis of CWD in white-tailed deer.
- Epidemiology of chronic wasting disease: detection of PrP<sup>res</sup>, shedding, and environmental contamination.
- Evaluation of third eyelid biopsy for detection of chronic wasting disease infection in mule deer.
- Survey for chronic wasting disease in cottontail rabbit populations.
- Leuprolide as a contraceptive agent in female elk: determination of effective minimum dose.
- Evaluation of GnRH-PAP as a chemosterilant in captive mule deer. I. Effects on animal health.
- Experimental evaluation of a vaccine for clostridium perfringens type A in captive bighorn sheep (*Ovis canadensis*) and captive mule deer (*Odocoileus hemionus*).
- Training personnel for tonsil biopsy for chronic wasting disease in mule deer.
- Field Immobilization Training.

#### Facility Improvement Projects:

A variety of scheduled and unscheduled maintenance and repair activities were necessary to support facility operation and ongoing research programs. Highlights include construction of new animal holding facilities and restoration of the metabolic cages to accommodate CWD epidemiology research. The mountain lion holding facility was also completed with the exception of the scale and squeeze/treatment area, as we are still in the design phase on this portion of the project. Additional

funding assistance from the Wildlife Health Laboratory (WHL) permitted installation of a separate perimeter fence around WHL. The new fence will allow easier access to the laboratory, while controlling traffic into and out of the animal holding facility.

Additional facility modifications include allocation of funding from the capitol construction team to replace the main hay barn. The barn is scheduled for replacement in the summer of 2005, and will be relocated to a central site with better access. The engineering office assisted with the development of electronic site maps of the facility. The site maps show exact locations for buildings and animal holding facilities, as well as locations for all known utilities. The maps will be updated periodically as facility construction and modifications occur. In addition, the FWRP landowner; Colorado State University approved an easement for the Northern Colorado Water Conservation District (NCWCD) to install a 68 inch water pipeline through the center of the facility (north to south). The easement was approved by the CDOW legal staff and included stipulations to maintain the perimeter fence, vehicle access, and keep all excavated soil within the perimeter of FWRP. The installation process partially disrupted FWRP management activities for a six week period, but resulted in an upgraded road system, and replacement of existing windrows with 200 five gallon potted trees and shrubs. NCWCD donated three culverts, built up the road system with excavated dirt, and added road-base which will allow for better water run-off, and should reduce road maintenance costs in the future.

Facility maintenance and construction projects were prioritized based on animal welfare concerns and anticipated research needs. Table 3 summarizes the completed, current, and on-going facility construction maintenance projects for the period.



Table 3. Facility Improvement Projects

<b>Project</b>	<b>Status</b>	<b>Details</b>	<b>Completion Year</b>
1. CWD Therapy Pens	Completed	Split 2 pens into 4, Construct 3 new shelters, add 1 automatic water (2 others included in east side plumbing upgrades above)	2003/2004
2. Travel Trailer Installation	Completed	Prepare 3 sites, winterize, hook into electric, water, septic, and purchase propane tank for one, electric and water hook-up for the others, electrical and furnace repair for the third, and misc. repairs for housing, office, and lab space	2003/2004
3. WHL and FWRF Parking Area Improvements	Completed	Add road base, gravel, landscaping timbers to expand and improve parking areas	2003/2004
4. D3-6 Feed Area Enclosures /catch areas	Completed	Re-set poles, replace range wire and snow fence with Hog panels in MD feed areas and catch areas	2003/2004
5. Electrical Upgrades	Completed	Increased power needed for expanding facilities- East and West sides, West side upgrades provided by WSVL	2003/2004
6. New Tool and Equipment Shed	Completed	Provided by NCWCD to replace shed demolished for water pipeline construction	2003/2004
7. E4 Site Clean-up	Completed	Remove 6 top inches of soil, saturate with 20% bleach soln., add 2 inches of road-base to lambing area	2003/2004
8. Lion Facility Walk-in Freezer /Cooler	Completed, Engineering request approved	Add a 10 x 10 cooler, and 10 x 10 freezer unit to the mountain lion complex	2003/2004
9. Road Improvements	Completed	The road system was built-up with extra dirt to enhance water run-off, 4 new culverts, time and equipt. to build up road system and install culverts were donated by NCWCD	2003/2004
10. FWRF Electronic Site Maps	Completed	Generated electronic site maps from an aerial photo, with all utilities, animal holding facilities, and structures	2003/2004
11. WHL Water Shut-off Valve	Completed	Valve was added to allow shut off the pen and necropsy lab water, while still providing water to the lab	2003/2004
12. Office Septic Pipe Repair	Completed	Emergency repairs to a cracked septic pipe	2003/2004
13. WHL Perimeter Fence	Completed	Construct a new perimeter fence around the lab to allow access to the lab without compromising the animal holding facility perimeter fence	2003/2004

<b>Project</b>	<b>Status</b>	<b>Details</b>	<b>Completion Year</b>
14. Emergency Fawn Rearing Shelters	Completed	Dairy calf shelters purchased and installed in waterfowl pen, fences modified to accommodate fawns	2003/2004
15. Equipment storage slab	Completed	Pour a concrete slab to store tractor and bobcat attachments out of the mud	2004/2005
16. DOD study Facilities	Completed	Convert E7 into 4 pens, add 6 automatic waters, construct 2 alleys, repair N. alley and Dig. Cage ramps, add drainage ditch, double fencing, refurbish the metabolic cages Some materials and labor donated by WSVL	2004/2005
17. Trailer Water Shut-Off Valve Replacement	Completed	Replace leaking water shut-off valve to FWRP travel trailer	2004/2005
18. Mountain Lion Facility	Completed	Utilities, concrete block building, 50 x 60 foot outdoor pen, shift containment system, and 4 indoor dens	2004/2005
19. New roofs/repair structure on old feed-sheds and animal shelters.	On-going project	Approx. ¼ of the old structures and roofs on the facility have been replaced in the last 2 years using treated lumber and long lasting roofing materials	Began 2000/2001, as needed
20. Add additional animal shelters	On-going project	Construct additional shelters in pens with heavy stocking rates. (36 ungulate pens on the facility)	Began 2001/2002, as needed
21. Road Maintenance	On-going project	Road grading and upkeep	As needed
22. Paint old building exteriors	On-going project	Now using CCA treated lumber or metal siding for repairs & building replacements to reduce the amount of painting necessary in the future.	Old structures are on a painting schedule every 3-5 years
23. Repair/replace latches, and broken or water damaged alley-way boards	On-going project	Now using CCA treated lumber for all repairs	As needed
24. Replace walk thru alley gates	On-going project	Replace old gates as necessary	As needed
25. Replace old visual barrier fencing and utility wire on metal gates	On-going project: most of the old material has been replaced, but this project is on-going due to animal and environmental damage	Old snow fence and construction fence replaced and moved to the outside of the paddock fence (except interior fences), utility wire is systematically being replaced with horse-fence	Began 2001/2002, as needed
26. Animal holding fence upgrades, and repairs	On-going project: rotten posts have been replaced, double fences constructed	Replace old range fence and V-mesh, as well as electric fencing in pens that house deer, Construct double fences as required by CWD protocols	Began 2002/2003, As needed

<b>Project</b>	<b>Status</b>	<b>Details</b>	<b>Completion Year</b>
27. Construct artificial refuge areas inside pens for neonates and adults	On-going project: completed for all new east side paddocks, maintain existing, construct new	Construct single and L-shaped, refuge areas to provide refuge and shade, construct hog panel seasonal enclosures to promote vegetation growth in the spring	Began 2002/2003, As needed
28. Add windscreen to west and south facing fence-lines	On-going project	Provide additional shaded areas for animals, and maintain existing	Began 2002/2003, As needed
29. Mowing and weed control	On-going project	Seasonal mowing and manual, chemical noxious weed control	As needed
30. WHL maintenance	On-going project	Provide maintenance assistance to WHL, and support for initial lab construction	Began 2002/2003, As needed
31. Unscheduled miscellaneous emergency facility repairs	On-going project	Emergency repairs to structures, animal holding facilities, perimeter fence, automatic waters, utilities, etc...	As Needed

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Prepared by \_\_\_\_\_

Tracy R. Davis, Wildlife Technician



### JOB PROGRESS REPORT

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

Period Covered: July 1, 2003 - June 30, 2004

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.

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### 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. Estimates of spring and fall turkey, spring snow goose, sharp-tailed and sage grouse, chukars, ptarmigan, Abert's squirrels, and general small game harvest were computed from survey data, 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 95/98/NT/2000/ME/XP program. A User's Manual was provided to terrestrial biologists on CD and also distributed via the WWW at <http://www.cnr.colostate.edu/~gwhite/deaman>.
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 use of lek counts to index prairie grouse populations was published in the Wildlife Society Bulletin: Walsh, D. P., G. C. White, T. E. Remington, and D. C. Bowden. 2004. Evaluation of the lek count index for greater sage-grouse. Wildlife Society Bulletin 32: 56-68.
5. A paper on the effects of early season hunter numbers on elk movement was published in the Journal of Wildlife Management: Vieira, M. E. P., M. M. Conner, G. C. White, and D. J.

- Freddy. 2003. Effects of archery hunter numbers and opening dates on elk movement. *Journal of Wildlife Management*. 67:717-728.
6. A paper discussing the implications of the GMU 10 special mule deer surveys was accepted for publication 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. Graham, J. H. Ellenberger, and C. H. Wagner. 2004. How many mule deer are there? Challenges of credibility in Colorado. *Wildlife Society Bulletin*. In Press.
  7. 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. 2004. Effect of limited antlered harvest on mule deer sex and age ratios. *Wildlife Society Bulletin*. In Press.
  8. A paper on the survival and recruitment of peregrine falcons was accepted for publication 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 Colorado peregrine falcon population. *Journal of Wildlife Management*. In Press.
  9. A paper on the estimation of the area of black-tailed prairie dog colonies in eastern Colorado was submitted to the *Wildlife Society Bulletin*: White, G. C., J. R. Dennis, and F. M. Pusateri. 2004. Area of black-tailed prairie dog colonies in eastern Colorado. *Wildlife Society Bulletin*. Submitted.
  10. A paper on methodologies to obtain more rigorous population monitoring data was submitted to *Wildlife Research*: White, G. C. 2004. Correcting counts: techniques to de-index. *Wildlife Research*. Submitted.
  11. A paper evaluating methods of estimating the impact of harvest on survival rates was submitted to *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*. Submitted.
  12. A paper on the procedures to monitor swift fox populations in eastern Colorado was submitted to 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*. Submitted.
  13. 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.
  14. A graduate research project (M. S.) to develop a sage grouse population model, using North Park sage grouse data to develop parameter estimates, was continued. The graduate student is Kristen Strohm.
  15. A graduate research project (M. S.) To evaluate line transect methodology for estimating pronghorn populations in eastern Colorado was initiated. The graduate student is Aaron Linstrom, and the project is in addition to his full-time duties as a terrestrial biologist with CDOW.
  16. 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 initiated. The graduate student is Paul Conn.
  17. 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.
  18. Development of the design of a monitoring system for swift fox in eastern Colorado was continued. This effort is in cooperation with Francie Pusatari and Darby Finley.

19. A workshop on use of the DEAMAN software for data entry, data summaries, and population modeling was presented to CDOW Terrestrial Biologists on May 20, 2004. A revised edition of the DEAMAN User's Manual was provided on a CD.

**JOB PROGRESS REPORT  
CONSULTING SERVICES FOR MARK-RECAPTURE ANALYSES**

G. C. White

**P. N. OBJECTIVES**

Extend existing methods to better provide rigorous population monitoring systems.

**SEGMENT OBJECTIVES**

1. Extend a mark-recapture monitoring scheme to estimate population sizes with inadequate data per site to estimate encounter probabilities.
2. Contrast line transect distance sampling approaches with mark-recapture approaches for monitoring populations with inadequate data per site to estimate encounter probabilities.

**ABSTRACT**

One of the most pervasive uses of indices of wildlife populations is uncorrected counts of animals. Two examples are the minimum number known alive from capture and release studies, and aerial surveys where the detection probability is not estimated from a sightability model, marked animals, or distance sampling. Both the mark-recapture and distance sampling estimators are techniques to estimate the probability of detection of an individual animal (or cluster of animals), which is then used to correct a count of animals. However, often the number of animals in a survey is inadequate to compute an estimate of the detection probability, and hence correct the count. Modern methods allow sophisticated modeling to estimate the detection probability, including incorporating covariates to provide additional information about the detection probability. Examples from both distance and mark-recapture sampling are presented to demonstrate the approach.

**RESULTS AND DISCUSSION**

The practice of using raw counts of animals as an index of the population size is one of the most pervasive uses of indices in wildlife management (Anderson 2003; Engeman 2003; Anderson 2001). Two examples include aerial surveys where no probability of detection is used to correct the count of observed animals, and the use of the minimum number known alive (MNKA) in animal (particularly small mammal) trapping studies (Slade and Blair 2000; McKelvey and Pearson 2001). These counts are known to be biased estimates of population size, and when used as an index, are assumed to be proportional to population size. These uses of uncorrected counts are some of the most perilous uses of an index in the practice of wildlife management because this assumption of proportionality is seldom verified, and is often false.

Nichols (1992) appealed to researchers to incorporate capture-recapture estimators into small mammal studies. However, various reasons are given to explain why indices are used in place of more rigorous capture-recapture estimators. The most common reasons (Slade and Blair 2000) include fear of violating assumptions basic to mark-recapture models (Nichols and Pollack 1983), failure to recognize that some of these models are relatively robust to heterogeneity of capture probability and trap response (Carothers 1979), mistaken belief that MNKA suffers less than other models from problems of differential probabilities of capture and survival when capture probabilities are high (Nichols and Pollack 1983; Montgomery 1987), and prevalence of protocols involving fewer than the 5 to 7 trapping occasions recommended for model selection and population estimation (Otis et al. 1978).



McKelvey and Pearson (2001) found that 98% of the samples collected in studies published from 1996 through 2000 they reviewed were too small for reliable selection among models of population estimation. However, their results do not take into account improved model selection methodologies, and new software and estimators that allow combining data across multiple studies and/or sites to provide more reliable model selection and estimation of the nuisance parameters. Their results mainly reflect the capabilities of CAPTURE (Otis et al. 1978; White et al. 1982), a software package developed in the late 1970's. More recent developments are available. The purpose of this presentation is to present the advantages of modern methods of analysis that allow combining data from multiple studies into a type of meta-analysis. Although resulting estimates of population size may not be completely unbiased, these estimates will certainly have less bias than MNKA. As discussed by Eberhardt et al. (1999), status of endangered large-mammal populations may have to depend on indices of population trend, and such indices may be improved by using auxiliary variables. However, in this paper, I go beyond just trying to standardize the counts with auxiliary information, as done by Eberhardt et al. (1999), and present methods incorporating auxiliary covariates that provide estimates of the population size.

### Modern Methods

Correcting counts to produce estimates of population size. Estimators of population size based on counts of animals share a common form. A count,  $C$  is corrected for the detection probability,  $p$ , to give the population size. Because the detection probability must be estimated as  $\hat{p}$  (or otherwise  $N$  would be known), the result is an estimate of population size

$\hat{N} = \frac{C}{\hat{p}}$  (Nichols 1992). The standard methods used in wildlife studies to estimate  $p$  are mark-

encounter methods and distance sampling. Both these seemingly diverse methodologies perform the same function: to correct a count of animals by the probability of detecting an animal.

To illustrate, consider the simple Lincoln-Petersen estimator:

$$\hat{N} = \frac{n_1 n_2}{m_2} = \frac{n_2}{\frac{m_2}{n_1}} = \frac{n_2}{\hat{p}},$$

where  $n_1$  and  $n_2$  are the numbers of animals captured on occasions 1 and 2, and  $m_2$  is the number of animals marked on occasion 1 that are recaptured on occasion 2. Thus,  $\frac{m_2}{n_1}$  is an estimate of the capture

probability on the second occasion (Nichols 1992), because we know that  $n_1$  animals are available for capture on the second occasion, of which  $m_2$  were captured. For distance sampling, the estimate of density (Buckland et al. 1993) is:

$$\hat{D} = \frac{n\hat{f}(0)}{2LW} = \frac{n}{\hat{p}2LW} = \frac{n}{\hat{p}}/A,$$

where  $n$  is the count of animals,  $2LW$  is the area surveyed (both sides of the transect line of length  $L$  out to a strip width  $W$ ), and  $\hat{f}(0)$  is equivalent to  $1/\hat{p}$ . So, the right-hand side of the equation is just the

corrected count  $\left(\hat{N} = \frac{n}{\hat{p}}\right)$  divided by the area counted to give density. The sightability correction

models of Samuel et al. (1987) also use an equivalent approach. The probability of sighting an animal is computed for each of the groups of animals sighted, and then the number of animals in the group is divided by the estimated sighting probability to estimate the number of animals under the observed conditions that were missed. When these estimates are summed across all groups, an overall estimate of population size is obtained. Although at first glance this estimator appears to be different than the forms shown above, in fact, it is exactly the same idea. Counts are corrected by an estimate of sightability.

However, because the sightability models of Samuel et al. (1987) and their extensions require first developing a model that is then applied to multiple surveys, the protocol deviates from what is the focus of this paper. That is, this paper centers on the idea of combining a number of sparse datasets into one analysis to achieve better inferences. Therefore, sightability models do not particularly fit into this approach, and so will not be discussed further here.

The take-home message of this section is that counts are corrected by some probability to achieve an estimate of population size. If this correction is the same when comparing results from two surveys, then comparing just the counts will result in the same proportional change. However, without verification of the assumption that the correction is the same for both surveys, erroneous results may ensue (Nichols 1992). Consider two counts of  $C_1 = C_2 = 100$ , but  $\hat{p}_1 = 0.5$  and  $\hat{p}_2 = 0.25$ , resulting in  $\hat{N}_1 = 200$  and  $\hat{N}_2 = 400$ . Without knowledge of the detection probabilities, the erroneous conclusion that the population had not changed would have been made. Of course, the opposite situation can also occur. Suppose  $C_1 = 200$  and  $C_2 = 100$ , with  $\hat{p}_1 = 0.5$  and  $\hat{p}_2 = 0.25$ , resulting in both  $\hat{N}_1 = \hat{N}_2 = 400$ . Just comparing the counts results in the erroneous conclusion that the population has changed, when in fact, only the detection probability has changed. Thus, comparing counts is dangerous without knowledge of the underlying detection probabilities. In the next section, more advanced approaches to estimation of the detection probability are presented.

*Improved modeling of data to produce estimates.* Earlier approaches to estimation of the size of a closed population only used the information available from the data at hand, e.g., Otis et al. (1978). Program CAPTURE (White et al. 1982) produced separate analyses for each species, sex- and age-class, and trapping grid. However, newer software packages, such as Program MARK (White and Burnham 1999) allow the user to model parameters in user-defined models. As a result, the detection probability for a population estimator can be modeled with group-specific, time-specific, and even individual-specific covariates. These covariates provide additional information with which to improve the estimates of the detection parameters. With this kind of model building capability, multiple sparse (but related) datasets can be combined into models to generate more precise estimates of  $p$ . These estimates of detection parameters and population size do not necessarily have to be the same for each of the datasets included in the analysis. For example, suppose capture probabilities are related to habitat quality, with animals in high quality habitat having smaller home ranges, and hence less probability of encountering traps. The approach advocated here is to build a model of detection probabilities by combining the data from multiple study areas and using the information about habitat quality in the model. For example,

$$\text{logit}(\hat{p}) = \beta_0 + \beta_1 \text{Habitat Quality},$$

where logit is the logit transformation  $[\text{logit}(p) = \log\left(\frac{p}{1-p}\right)]$ . The result is a model predicting

capture probability as a function of habitat quality, where the parameters  $\beta_0$  and  $\beta_1$  are estimated from the data and the habitat quality values provided by the user. Instead of estimating a separate value of  $p$  for each of the study areas, and likely encountering problems with too small of sample sizes, the researcher obtains an estimate of  $p$  specific to each study area based on habitat quality.

Besides incorporating covariates into the model, less parameter-rich models that are still biologically realistic can be fitted to the observed data. A more extensive example is provided in White (2001), where models are combined across day- and night-time trapping occasions, gender and age-class. That example demonstrates the capability of additive models, where additive effects in the model (e.g., an effect representing the difference between day and night capture probabilities) provide differences, yet maintain a parallelism between the estimates across time or other categories. Additive models provide a useful alternative to the full multiplicative model. For example, suppose there are 5 trapping grids, each

trapped for 5 nights. The full multiplicative time-specific model would have  $5 \times 5 = 25$  parameters. In contrast, the additive model would still have 5 time parameters, but with only 4 additional parameters to represent the differences between study areas, resulting in only 9 parameters being estimated from the data. Hence, at the expense of possible bias, much improved precision of the estimates will be obtained.

Finally, modern approaches provide much more flexibility in exploring alternative models. With CAPTURE, it was all or none. Only 8 models from the  $M_{tbi}$  set were defined, and these were cast in concrete. Only 7 models of this set had estimators (with only 5 estimators in the original program). However, with Program MARK (White and Burnham 1999), all the likelihood models from CAPTURE can be reproduced, plus many additional possibilities are provided by variations of the original 8 models. Included in MARK are the mixture models of Pledger (2000) for modeling individual heterogeneity, and Huggins (1989, 1991) and Alho (1990) versions of the closed capture estimators that allow individual covariates to be used to model initial and recapture probabilities.

Thus, approaches available in MARK provide 3 areas of improvement to handle sparse mark-recapture datasets. First, covariates can be incorporated into the analysis, bring additional information. Second, flexible modeling structures can provide biologically reasonable models to combine sparse datasets. Third, more flexibility is provided to construct capture-recapture models of individual datasets.

Program DISTANCE 3.5 (Thomas et al. 1998; Buckland et al. 2001), and now updated to DISTANCE 4.1, provides similar capabilities for distance sampling data as does MARK for mark-encounter data. Data are presented to the program in strata, with stratum-specific estimates of density provided from detection probabilities estimated across strata. Although not as flexible at this time in terms of building complex models that incorporate covariates, these capabilities are forthcoming in newer versions of the program. The essence of the approach advocated here is currently available in DISTANCE – multiple datasets can be combined to estimate the sightability parameter, yet stratum-specific estimates of density are achieved.

Model selection methods. Another feature of modern methods is that the estimate from a single model is not accepted as the best estimate available from the data. Burnham and Anderson (2002) describe information-theoretic model selection methods, leading to model averaging, where estimates from multiple models are combined to obtain an estimate that is an improvement over estimates from single models. The traditional approach was to find the “best” model, and use that model to make inferences from the data. However, the process of sorting through the available models carries some baggage – multiple decisions are required to decide which model is most appropriate. As a result, a source of variation in the data analysis process is ignored – model selection uncertainty. Simulations have shown that the estimates and their confidence intervals from the “best” model do not perform as hoped (Burnham et al. 1995). In particular, confidence intervals do not cover the parameter value for the expected 95% with  $\alpha = 0.05$ .

Rather than accept the poor performance because of ignoring model selection uncertainty from using the “best” model, the model-averaging methodology provided by Burnham and Anderson (2002) incorporates the model-selection uncertainty into the estimates and associated confidence intervals. Further, the approach is more biologically satisfying. For example, who really believes that the “best” model for making inferences from a capture-recapture study is something simple like  $M_t$ ? Rather, we would all suspect some individual heterogeneity to be present, as in  $M_h$ . Yet, with the traditional approach of just making inferences from the “best” model, the individual heterogeneity aspect would be completely ignored if  $M_t$  was determined to be the “best” model. With model averaging, we incorporate information from all the models that have weight associated with them, with the information provided by each model proportional to its weight. The result is an estimate that reflects more accurately what we know from the data, and that a single model is inadequate for making inferences from the data.

A key part of model averaging is estimating the weight to be associated with each model. The information-theoretic approach presented by Burnham and Anderson (2002) is based on Akaike's Information Criterion (AIC). Without going into the mathematics (details are presented in Burnham and Anderson 2001; Burnham and Anderson 2002), the general idea behind AIC model selection is to rank models based on the trade-off between bias versus precision of the estimates (Figure 1). Simple models, i.e., models with small numbers of parameters, produce more precise estimates at the expense of potentially biased estimates. In contrast, complex models, i.e., models with large numbers of parameters, will produce generally unbiased estimates, but at the cost of poor precision. That is, the sampling variance of the parameter estimates from complex models will be large compared to simple model.

From the AIC value for each model, a weight is computed for each model. These weights are standardized to sum to 1, so that the weight of a model reflects the likelihood of the model. From these weights, the model-averaged estimate of the parameter across all the models considered is computed.

Program MARK includes information-theoretic (i.e., AIC) model selection criteria (White and Burnham 1999) and the capability for model averaging population estimates (White et al. 2001). Although DISTANCE includes AIC model selection, the capability to model average is not presently available. However, the calculations for model averaging are simple, and can be easily performed in a spreadsheet given the estimates, standard errors, and AIC values.

So what's the price for using the approach advocated here? For the user, likely a fairly steep learning curve must be climbed. More statistical and computer expertise is required to conduct the analyses described than with traditional approaches. Although likely an excuse, competent scientists will not let this reason keep them from applying better methodology to more fully interpret their data. Data are hard to come by, and deserve full treatment once acquired.

#### Quadrat sampling example

I now present an example of estimating the population size of the Mexican spotted owl in the Upper Gila Mountains Recovery Unit. Twenty-five quadrats 50–75 km<sup>2</sup> were sampled for owls with a 4-pass removal sampling scheme (Ganey et al. 1999). When an owl was detected through night-time calling, it was located the next day and leg banded to individually identify it. Recaptures were obtained when a marked owl was located during a latter pass. These capture-recapture data from banded owls on the 11 quadrats where owls had been banded and subsequently resighted were used to estimate  $p$ , the probability of capture on a given trapping occasion (Huggins 1989). To estimate  $p$ , a closed capture-recapture modeling procedure developed by Huggins (1989, 1991) that was implemented in Program MARK (White and Burnham 1999) was used. The goal was to estimate  $p$  as precisely as possible because the sampling variances of the  $p$ 's contribute to the sampling variances of the estimated  $N$ 's. In addition to  $p$ , the probability of recapture ( $c$ ) can also be estimated, adding an additional parameter to be modeled. In standard closed capture-recapture models, maximum likelihood estimation is used to estimate both  $p$  and  $N$ , simultaneously (Otis et al. 1978), i.e., the resulting estimates from standard closed capture-recapture models represent the joint maximum likelihood estimates. The Huggins models differ from the standard models in that only  $p$  and  $c$  are modeled with  $N$  being estimated as a derived parameter (i.e.,  $N$  is computed algebraically from  $p$ ). Thus, our initial efforts centered about modeling the capture-recapture data to obtain parsimonious estimates of  $p$ . The key point relevant to this paper is that no one quadrat had adequate data to estimate  $p$  and/or  $c$ . Data were pooled across quadrats to obtain these estimates of detection probabilities, and then used to generate an estimate of  $N$  for each of the quadrats.

To estimate  $p$ , 26 closed-capture models were run in program MARK. The notation used to describe these models follows Lebreton et al. (1992). In this set of models, the effects on  $p$  were modeled by sex, road access to the quadrat, occasion-specificity, and behavioral response to initial capture (i.e., inclusion of the recapture parameter  $c$  in the model). A bias-corrected version of Akaike's Information

Criteria,  $AIC_c$  (Burnham and Anderson 2002) was used to rank models with the best model having the lowest  $AIC_c$ . The best model was  $p = c_{T+roadless+sex}$ , which constrained  $p$ 's equal to  $c$ 's, and had a linear occasion effect ( $T$ ), an effect of roadless quadrats versus non-roadless quadrats and a sex effect on the  $p$ 's. The linear occasion, roadless and sex effects were all negative and different from zero ( $\beta_T = -0.350$ , 95% CI = -0.637, -0.063;  $\beta_{roadless} = -1.614$ , 95% CI = -2.742, -0.486;  $\beta_{sex} = -0.983$ , 95% CI = -1.764, -0.203). This model indicated that capture probabilities declined over occasions in a linear fashion, roadless quadrats had lower capture probabilities than roaded quadrats, and that females had lower capture probabilities than males. Rather than using the  $p$ 's solely from this model, Akaike weights were estimated for each model (Buckland et al. 1997; Burnham and Anderson 2002) which represented the likelihood of a specific model as the best model to explain this particular data set, relative to the other models examined in our set of models. Akaike weights were then used to derive a weighted mean estimate of capture probabilities ( $p_i$ ) (i.e., the  $p_i$  were "model averaged") for each occasion for each sex and within roaded and unroaded quadrats across all models (see Stanley 1998a, 1998b). These weighted estimates of  $p_i$  had estimated standard errors that included a variance component due to model selection uncertainty, i.e., which model was best for providing an adequate structure on the  $p$ 's (Buckland et al. 1997; Burnham and Anderson 2002). Thus, we ended up with 16 estimates of  $p$ , one for each of four occasions times two types of quadrats (roaded versus unroaded) and for each sex. Based on these estimates of  $p$ , a population estimate for the recovery unit was 2173 with SE 520. Had just the raw counts been used, the estimate would have been 1564 with SE 222.

This example illustrates an extreme case where each trapping grid (quadrat) contained so little information about detection probabilities that by individual quadrat, the researcher is left with no choice but to use the MNKA value. However, by combining these sparse data, useful estimates were obtained that corrected for the bias of MNKA.

## CONCLUSIONS

Sparse data need not be an impediment to correcting counts of populations to less biased estimates of population size. Modern methods incorporate information from auxiliary variables, build models from multiple sources of information, and build biologically reasonable models with fewer parameters than older approaches. Thus, past justifications of using counts as indices to population levels because of sparse data are no longer defensible. If biologists do not correct counts, we run the risk of drawing erroneous conclusions from our data, and generally losing credibility with our public critics.

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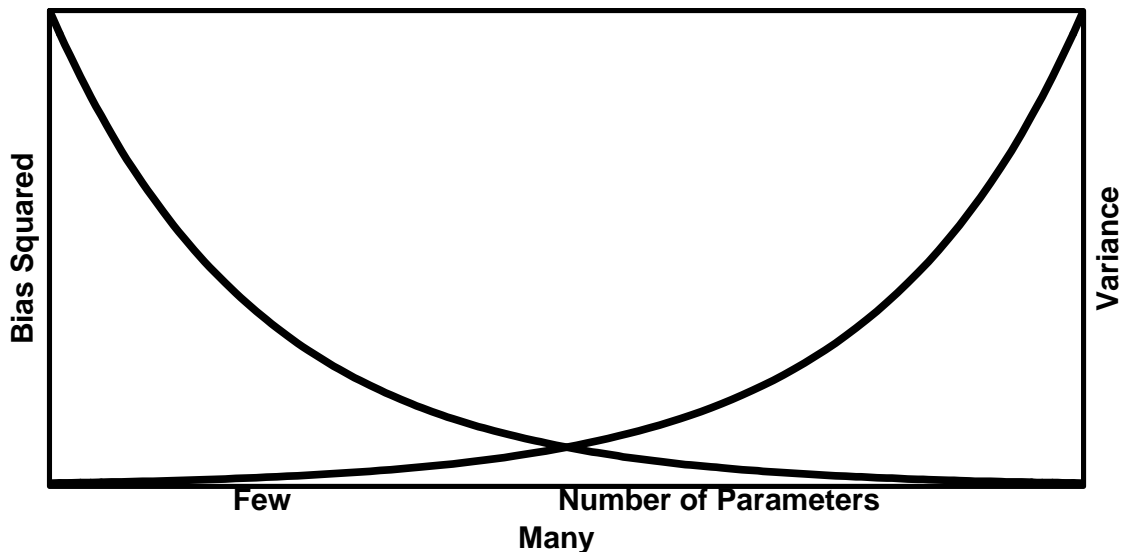


Figure 1. The trade-off between bias<sup>2</sup> and variance as a function of the number of parameters (from Burnham and Anderson 2002; 2001). Models with few parameters produce precise estimates that are biased, whereas models with many parameters produce less biased estimates, but imprecise.





Colorado Division of Wildlife  
Wildlife Research Report  
July 2003 – June 2004

### JOB PROGRESS REPORT

State of Colorado : Cost Center 3430  
Project No. \_\_\_\_\_ : Mammals Research  
Work Package No. 7210 : Research Support / Administrative Services  
Task No. \_\_\_\_\_ : Library Services

Federal Aid Project: N/A :

Period Covered: July 1, 2003 – June 30, 2004

Author: Jacqueline A. Boss

Personnel: Jacqueline 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:

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

1,922 Items of information delivered to Colorado Div. of Wildlife employees, cooperators, wildlife educators, and the public, resulting from requests and literature searches.

308 Items of information cataloged into the electronic and card catalogues, which including duplicates and additional volumes, expanded the Research Center Library inventory to 23,781 items.

726 Items of information entered into the electronic catalogue for the maintenance of the circulation system of the Research Center Library.

1,706 Items checked-out by Colorado Div. of Wildlife employees, cooperators, wildlife educators, and the public indicating satisfaction of library services.

1,430 Items of information delivered that are produced by the Colorado Div. of Wildlife employees, cooperators, wildlife educators, and the public. These items include publications (1,430 – From time to time duplicated books donated to our Library are also given to CDOW employees and are included in this number), research articles by CDOW personnel (650), and CDOW federal aid reports (183).

**JOB PROGRESS REPORT  
COLORADO DIV. OF WILDLIFE RESEARCH LIBRARY SERVICES**

Jacqueline A. Boss

**SEGMENT OBJECTIVE**

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

**SUMMARY OF SERVICES**

Maintain Electronic and Card Catalogues of all Research Library Holdings

308 is the 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 Colorado Div. of Wildlife Researchers and other employees.

726 is the 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.

\$211,167.94 is the "known value" of the 23,577 items in the Research Center Library collection as of June 30, 2004. 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 "known 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 "known 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 "known value" of the Library collection continues to grow month by month

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