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Pacific Northwest National Laboratory

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Investigation of Anatomical Anomalies in Hanford Site Mule Deer

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March 1997

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Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

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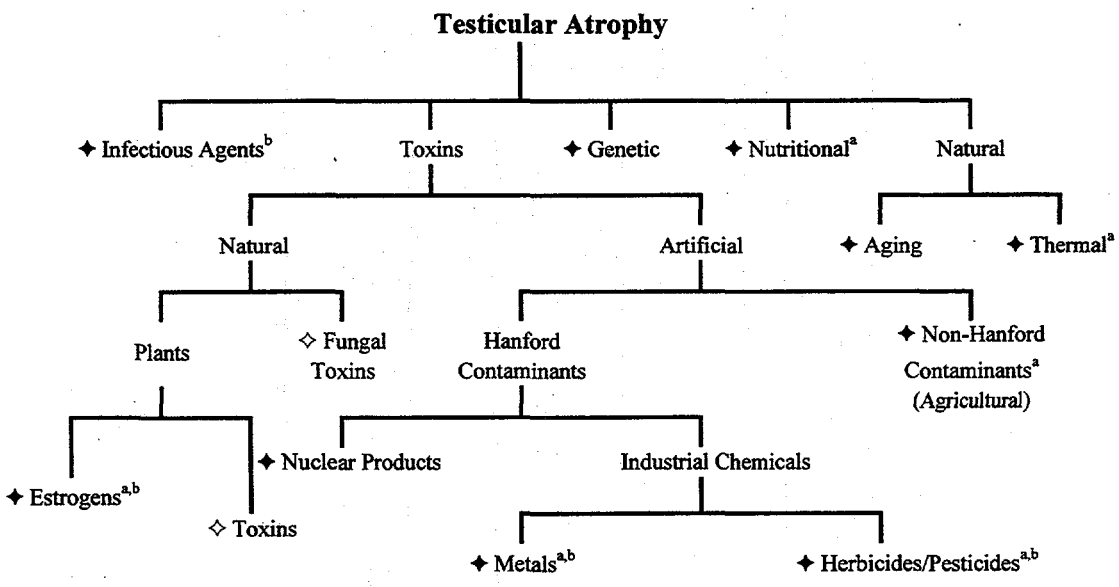
Executive Summary

Rocky Mountain mule deer (*Odocoileus hemionus hemionus*), common residents of the Hanford Site, are an important part of the shrub-steppe ecosystem as well as being valued for aesthetics and hunting. Because mule deer have been protected from hunting on the Site for 50 years, the herd has developed unique population characteristics, including a large number of old animals and males with either large or atypically developed antlers, in contrast to other herds in the semi-arid regions of the Northwest.

Hanford Site mule deer have been studied since 1991 because of the herd's unique nature and high degree of public interest. A special study of the mule deer herd was initiated in 1993 after observations were made of a relatively large number of male deer with atypical, velvet-covered antlers. This report specifically describes our analyses of adult male deer found on the Site with atypical antlers. The report includes estimates of population densities and composition; home ranges, habitat uses, and dietary habits; natural and human-induced causes of mortality; and the herd's overall health and reproductive status.

Examinations of adult male deer exhibiting unusually shaped, velvet-covered antlers were found to have permanently infertile, atrophied (shrunken as opposed to incompletely developed) testicles. The frequency of affected deer observed on Hanford was highly correlated to the frequency of older-aged animals found onsite and was difficult to compare with other more heavily hunted populations. Morphologic, physiologic, and clinical and pathological results showed essentially normal findings, but indicated the causative agent(s) act directly on testicular tissues. Figure S.1 shows a logistical flow diagram for potential causative factors responsible for deer exhibiting unusually shaped, velvet-covered antlers. Radiation, natural aging, infectious agents, and genetic factors were ruled out as primary potential causes. Other factors ruled out as likely causative agents that could warrant further field investigations were thermal stress, nutritional stress, plant estrogens, heavy metals, and herbicides/pesticides/insectides (Hanford and/or adjacent agricultural areas). Essentially no data were gathered to examine plant or fungal toxins, which could be causative agents.

Population-level results indicated that the Hanford Site herd contained a relatively large proportion of male deer, primarily as a result of the lack of hunting pressure and mild winters. Fawn production on Hanford was low. Deer were most abundant along the Columbia River (approximately 330 animals) and on the western portions of the Fitzner/Eberhardt Arid Lands Ecology (ALE) Reserve and were lowest on the 200 Area plateau to the southern border and the eastern portions of ALE. Deer diets and movements indicated a high dependency on riverine habitat, sagebrush, and the sand dunes habitat. The data also showed that radio-tagged deer avoided buildings, the white bluffs, basalt outcrops, cheatgrass/bunchgrass, and rabbitbrush/bunchgrasses land cover types.



✦denotes endpoints which have been eliminated as likely primary causes.

◇ denotes endpoints that have not been eliminated as likely primary causes.

[a] warrants field research.

[b] indicates laboratory studies are required.

Figure S.1. Potential Causative Agents of "Cactus Bucks" on the Hanford Site

Acknowledgments

This project was funded by the U.S. Department of Energy (DOE). Battelle Memorial Institute operates Pacific Northwest National Laboratory (PNNL) at the Hanford Site in south-central Washington for DOE under Contract DE-AC06-76RLO 1830. Analytical costs for radiological analyses were provided by PNNL's Surface and Environmental Surveillance Program (SESP).

Because of the nature of this study, it involved many persons, at some level or another. Special thanks go to Steve Tolle (Pathfinder Inc.) and John Musser for rotor-wing aircraft support during the deer capture events. Randy Hein (DVM) provided technical assistance during field surgeries and other capture-related sample collection efforts. For assistance with field data collections and deer capture events, we wish to thank many other individuals, including Rhett Zufelt, Steve Colvin, Alan Leary, Mike Houser, Corey Duberstein, Andrea Smasne, Cora Singleton, Mark Briggs, Lauri Hanauska, Mead Klavetter, Seji Karki, Jeff Marco, Nick Miller, Tracy Feldman, Jared Gratz, Jeffrey Stocum, Michael Blanton, Jennifer Sandoval, Beckeye Stanton, Sara Greiner, and Christopher Auger, Amber Alford. Although for many of these individuals, the highlights of field work such as an exciting deer capture were to be savored, much of their work involved long days of locating radio-equipped deer and watching a selected animal until it defecated. Had these individual's spirits not been so robust to monotony, much valuable data would have been missed. Personnel consistency is extremely important and we would like to give special thanks in this area to Rhett Zufelt who persisted throughout the venture. Rhett also contributed greatly by providing geographical positioning system and geographic information system (GPS/GIS) interface capability to illustrate this study

In addition, the authors wish to thank PNNL's Flight Aviation Safety Committee for assisting in aircraft operation setup and ensuring that much of this work could be performed under current DOE safety guidelines. Much of this task, as well as into deer capture events, animal movement analyses, and population size estimates, would not have been possible without their assistance. We also would like to thank Lockheed International Company (LITCO) and Hilsboro Helicopter for their efforts during the population estimates.

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1.0 Introduction

We studied Rocky Mountain mule deer (*Odocoileus hemionus hemionus*) on the 1,450 km^2 Hanford Site in south-central Washington from 1993-1996 as part of Pacific Northwest National Laboratory's (PNNL) Wildlife Resources Monitoring Project. Analytical support for the study was provided by the Surface Environmental Monitoring Project. Because mule deer have been protected from hunting on the Hanford Site for 50 years, the herd has developed unique population characteristics, including a large number of old animals and large-antlered males, in contrast to other herds in the semi-arid regions of the Northwest. Mule deer are of interest to wildlife management and contaminant monitoring programs because they serve as an indicator of environmental conditions of Hanford Site operations and provide useful information for current environmental clean-up efforts (Eberhardt and Cadwell 1983).

Studies of Hanford Site mule deer have been conducted since 1991. Routine monitoring of the herd along the Columbia River in 1991 and 1992 first revealed the atypical, velvet-covered antlers and abnormally developed testicles in 22% of adult males captured. Bucks exhibiting this condition also were observed while radio-tracking male deer in 1992 and 1993. Although the objectives and subsequent results from the 1991 and 1992 mule deer studies have been documented (Tiller et al. 1995), this report also includes observations and mortality data collected during that time.

1.1 Study Objectives

This report provides results of our 1993-1996 field study conducted specifically to examine the patterns of testicular atrophy in the Hanford Site herd and describes the ecology and current status of the herd. Specific study objectives were to:

- examine an atypical condition in some male deer
- estimate deer population densities and composition
- determine natural and human-induced causes of mortality
- determine deer home ranges, habitat uses, and dietary habits
- evaluate the overall condition and health of deer on the Hanford Site
- examine reproductive status of the herd.

To gather information on testicular atrophy patterns, we captured deer at several locations onsite to collect blood, tissue, and organ samples; conducted roadside surveys as well as fixed-wing and helicopter surveys using advanced technologies, and radio-equipped deer in the study area. Laboratory analyses were conducted of blood, tissue, and organ samples.

1.2 Study Area

We studied deer along an approximately 200 km^2 portion of the Hanford Site bordering the Columbia River in Benton and Grant counties, Washington (Figure 1.1). In general, the area is characterized by shrub-steppe vegetation dominated by big sagebrush (*Artemisia tridentata*) and Sandberg's bluegrass (*Poa sandbergii*) (Daubenmire 1970; Downs et al. 1993), with approximately 16 cm of annual precipitation (Hoitink and Burk 1994). The climate consists of hot dry summers and relatively cool winters when the bulk of annual precipitation occurs.

For comparative purposes, the study region was divided into north and south study areas, as shown in Figure 1.1. The southern area generally is unaltered by Hanford Site-related activities and is characterized by sand dunes, abandoned farm fields, and early successional shrub-steppe habitat recovering from a large wildfire in 1985. Plant communities in the dunes region are dominated by rabbitbrush (*Chrysothamnus* spp.) and bitterbrush (*Purshia tridentata*) (Downs et al. 1993). The northern study site contains six inactive nuclear production reactor sites, abandoned agricultural fields, and scattered patches of shrub-steppe habitat.

The Columbia River supports riparian habitat and riverine islands commonly used by resident mule deer. Shoreline vegetation along the Hanford Reach consists of a narrow zone of broad-leafed deciduous trees and shrubs intermingled with a variety of perennial grasses and forbs (Sackschewsky et al. 1992; Downs et al. 1993). The riparian zone tends to remain green throughout the hot dry summer months.

1.3 Report Contents

Section 2.0 of this report details methods and materials used in this study. Results related to the study objectives outlined above are included in Section 3.0. Conclusions and recommendations of the 1993-1996 study are presented in Section 4.0. Section 5.0 includes reference citations. Appendices A-F provide supporting data for the project.

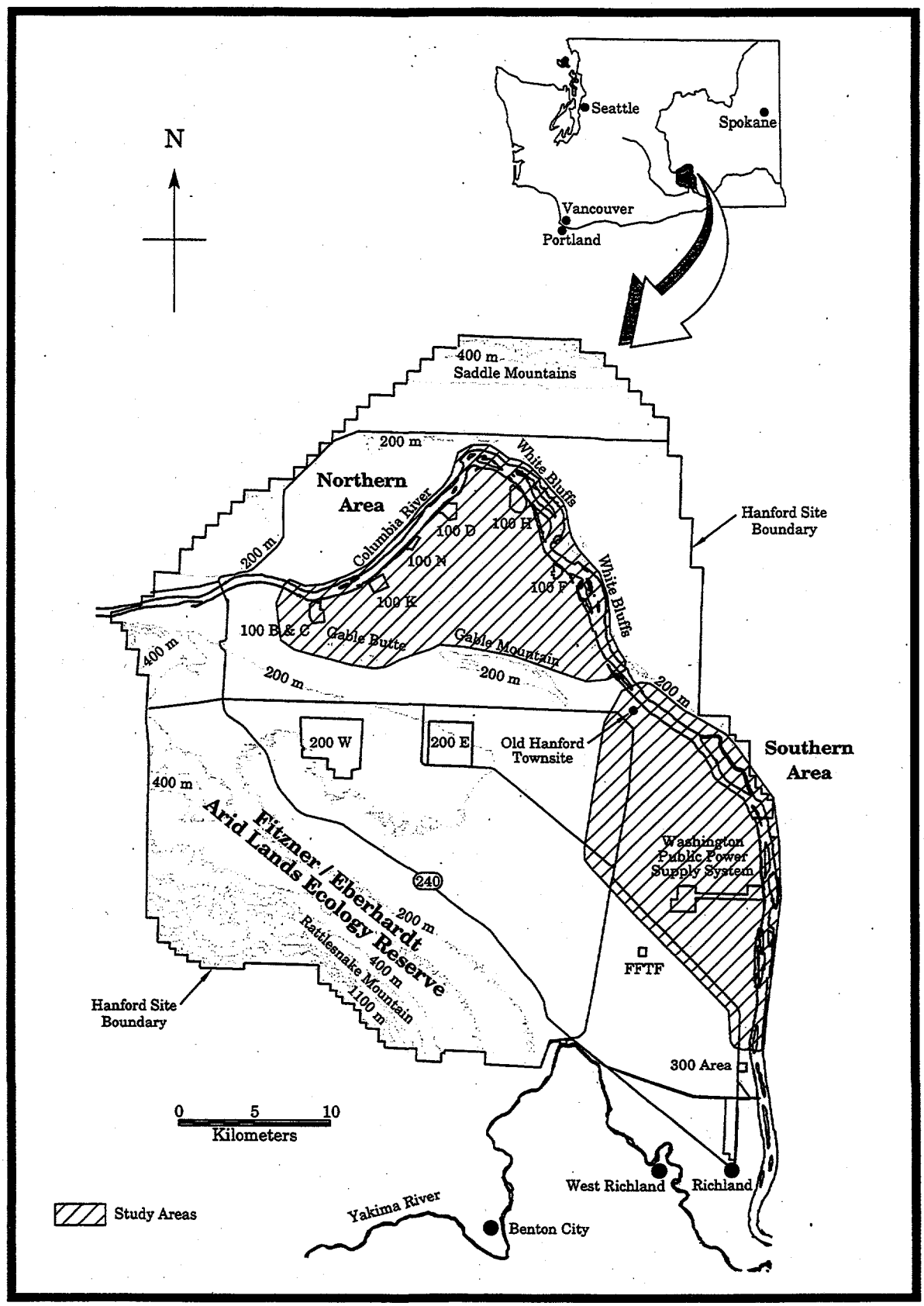


Figure 1.1. Hanford Site and Location of Northern and Southern Study Regions

2.0 Materials and Methods

This section provides details of field and laboratory techniques used in this study for deer capture events; roadside, aircraft, and radiotelemetry surveys, using advanced technologies, and microscopic, physiologic, and chemical analyses.

2.1 Animal Capture and Handling

Fifty-four mule deer were captured in drive nets (Beasom et al. 1980) at several locations along the Hanford Site bordering the Columbia River in February and March 1992 and 1993. In 1994, we captured an additional 21 male deer using a CODA net gun (CODA Ent., Mesa, Arizona) fired from a hovering helicopter. The captured animals were slung in a cargo net for transport to staging areas where animals were processed. Chase and handling time for these animals ranged from 1 to 3 min and 20 to 45 min, respectively. For all deer captured, we measured incisor 1 lengths, estimated age, noted general health, collected hair and fecal samples, fastened a solar-powered transmitter (Advanced Telemetry Systems, Isanti, Minnesota) to the ear of adult males, and when available, collected antler samples for radiological analyses. We removed a canine tooth from 29 males and submitted them to Matson's Laboratory (Milltown, Montana) for age determination by cementum annuli analysis (Erickson and Seliger 1969).

All animal capture, handling, and collection activities described in this report were carried out in compliance with PNNL's Animal Care Committee (10875/I-7) and the Washington Department of Fish and Wildlife Scientific Collection Permitting Processes (#WM-0038).

2.2 Population Characteristics

Materials and methods used to determine age and sex ratios, pregnancy rates, age distributions, and survival and mortalities of the Hanford herd are described below. The non-parametric Multi-Response Permutation Procedure (MRPP) was used to detect significant differences between selected data sets (Mielke 1991). Confidence intervals ($\alpha = 0.05$) were calculated for some of the data, and standard error (SE) were computed for virtually all data sets.

2.2.1 Sex and Age Structure

Buck:doe:fawn ratios were determined from roadside surveys conducted during 1993, 1994, 1995, and 1996. These ratios were observed in deer that reside along the Columbia River during the pre-hunting period (July - Sept) and post-hunting period (Dec - Feb). The exact age (±1 year) of all males captured in 1993 and 1994 was determined by cementum annuli analysis.

Age data were collected during the 1993 and 1994 deer captures (n = 29) and grouped to reflect the age of each animal at the time of initial capture. This was done so we could examine the "snap shot" age distribution of male deer on the Hanford Site.

2.2.2 Pregnancy Rates

Thirteen female deer were captured on the Hanford Site in February 1993, shortly after the mating period, and fitted with radio-collars. Pregnancy rates were determined from blood samples drawn from these female deer and assayed for pregnancy specific protein-B (Sasser 1986; Wood et al. 1986; Hein and Bracken 1991).

2.2.3 Fawn Production

Fawn survival rates were estimated systematically from herd composition counts and during weekly radiotracking of radio-collared does with fawns. In addition, 10 radio-equipped does were followed in 1991 and 20 in 1992 (from early July through September) to monitor postnatal survival of their fawns.

2.2.4 Age-Specific Survival

Adult and age-specific survival rates were estimated from mortality rates observed throughout the radiotelemetry activities conducted during the study period.

2.2.5 Adult Mortalities

Mortality rates and causes of mortality were determined from mortalities observed with radio-equipped deer. In 1994, 21 male deer residing near the Columbia River were captured, radio-tagged, and released for future monitoring purposes. One animal lost its radio transmitter within the first month after capture, but remaining deer were systematically tracked by air and observed on the ground through the 1994 hunting season.

2.3 Population Size Estimates

To estimate deer population densities, the Hanford Site was stratified into three regions: 1) Columbia River, 2) inner Hanford Site, and 3) east and west portions of the Fitzner/Eberhardt Arid Lands Ecology (ALE) Reserve (Figure 2.1). These areas were determined based on movements by radio-transmitted deer and PNNL staff knowledge of general deer densities on the Site. The number of mule deer residing in the river region was estimated using three different mark-resight techniques and Forward Looking Infrared (FLIR) technology, as described below. The relative abundance of deer residing on ALE and in the inner portions of the Site was estimated by counting the density of fecal pellet groups found there (Figure 2.1).

2.3.1 Mark-Resight

Deer mark-resight data for the 200 km^2 river region were collected during systematic road surveys in 1995 and evaluated using the following mark-resight models with a software program developed by White (1995): 1) joint maximum likelihood estimator from a hypergeometric distributions (JHE) developed by White (1993), 2) Minta-Mangel bootstrap estimator (Minta and Mangel 1989), and 3) Bowden's estimator (Bowden 1993).

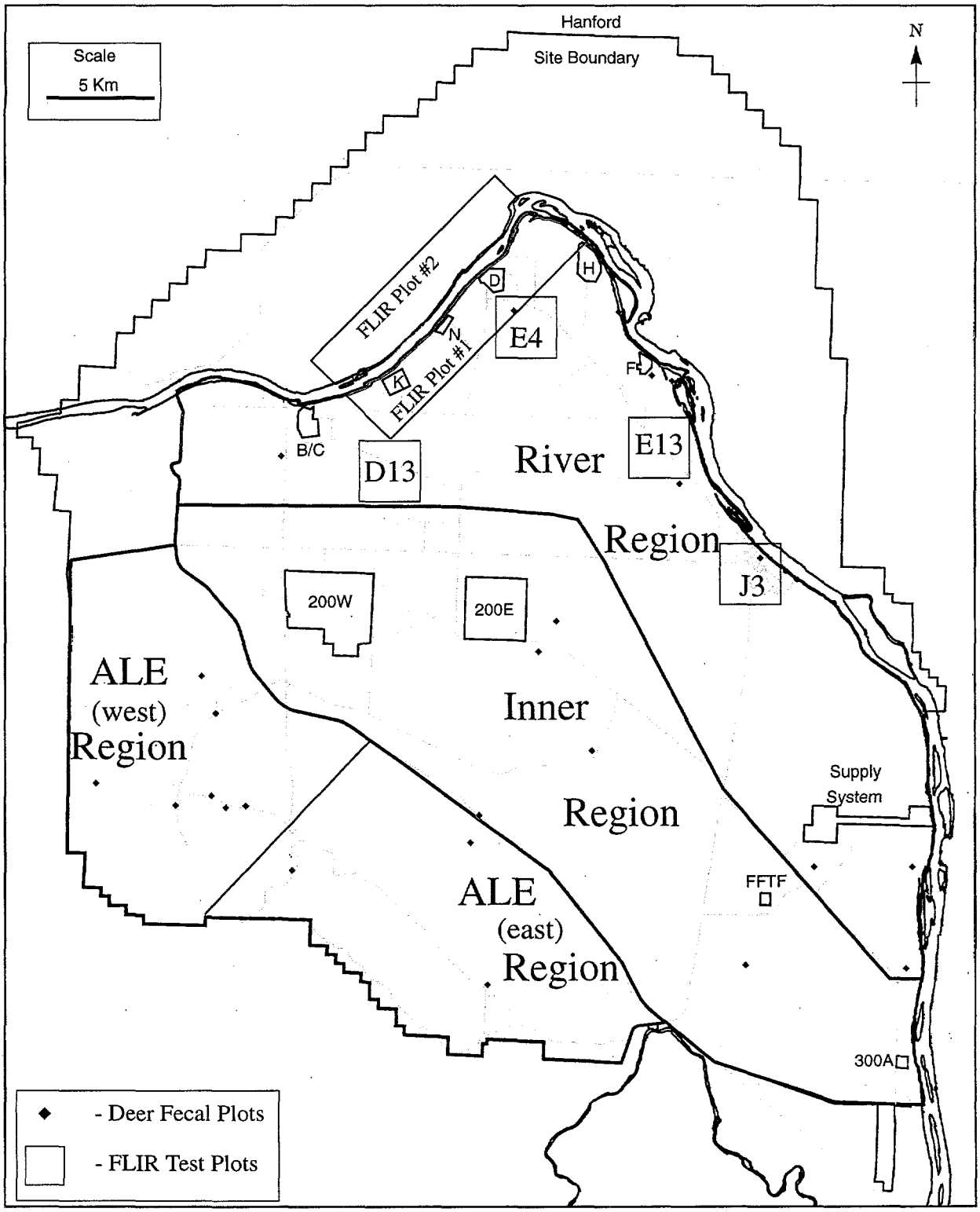


Figure 2.1. Site Delineation for Population Density Estimates, Locations of FLIR Test Plots, and Survey Regions

The JHE was calculated using the closed population model, which assumes all marked animals are in the area being surveyed. This method also assumes equal sighting probabilities and that no animal is observed or counted twice during any given survey occasion. A detailed discussion by Neal et al. (1993) examines the robustness of JHE when various assumptions are not met. Bowden's estimator allows for heterogeneity in animal sighting probability and a geographically "closed" study area. The resulting estimate approximates the total population based on the entire study area. The Minta-Mangel estimation also allows for heterogeneity in animal sighting but is derived based on the assumption that the same animal may be counted twice during a particular survey (Minta and Mangel 1989). For this study, the known number of marked animals in the study region was accurately determined because white-collared females (marked animals) had active radio-transmitters. All living white-butyl radio-collared females were located by fixed-wing telemetry just before the field survey period.

2.3.2 Forward Looking Infrared Technology

We estimated deer densities in the river region using a helicopter equipped with a high-infrared camera (FLIR 2000) and a global positioning system (GPS). The pilot, an experienced FLIR operator, and one PNNL staff member flew over two selected 36 km^2 test plots along the Columbia River and four other 3 km^2, plots near the river to evaluate the technique and record data. Deer FLIR surveys were flown in March and September 1995 from dawn until 0900 hr when ground temperatures were cool. Four test plots were flown systematically throughout the morning to examine changes in the FLIR's detectability of deer.

During the FLIR surveys, we located each plot using a GPS system, then used the FLIR camera to survey the plot for deer. The helicopter retained an altitude approximately 300 m above ground level. Generally, the flight path for each plot survey began at the southeast corner of the plot and continued to the northeast corner, the northwest corner, the southwest corner, and then back to the southeast corner. The pilot used a map we provided showing universal transmercator (UTM) coordinates to locate corner points of the survey plots. Flights continued until test plot results demonstrated less than 100% detection of animals. Two ground observers were stationed on the ground at the four plots near the river during the FLIR survey to count the number of deer actually present within the plots. Because these plots were in rather small and in open areas, we felt confident about the number of deer determined to be within the plots by ground observers.

2.3.3 Fecal Pellets

We estimated the relative abundance of deer residing within ALE and the inner portions of the Hanford Site by counting the number fecal pellet groups found within several 3-m radius plots (five plots within the inner region, five on ALE (west), and three on ALE (east). Fecal pellet group densities also were determined on nine plots located within the 150 km^2 river study region so we could use the results to estimate the total number of deer residing on the entire Hanford Site. In light of time and budget constraints, no deer pellet plots were established on the North Slope.

All fecal pellet plot sites were located in October 1994 by randomly selecting an area in vegetation cover types used indiscriminately by the deer (see use vs. availability). We then drove a metal T-post labeled with the respective plot site identification into the ground for a permanent marker. Data collection involved locating the center of the 3-m radius plots with a GPS from coordinates taken when the plot

sites were established. Using a metric tape, two or three persons walked around the T-post at 1.5-m spacing intervals. Pellet groups (five pellets or more clustered) were recorded by one observer and then removed from the plot to leave the plot free of pellets in preparation for the next 6-month count. If the area contained elk scat, it was noted then removed from the plot.

2.4 Deer Movements and Home Ranges

We evaluated deer movements and home ranges based on relocation data collected by fitting animals with collars containing radio-transmitters. A total of 31 female deer and 32 male deer were captured and their subsequent movements monitored via the radio-collars. We attempted to radio-track these animals weekly. Approximately 80% of the surveys were conducted from fixed-wing aircraft and 20% from the ground.

Deer home ranges and sub-population intermixing were evaluated based on radio-telemetry data. Animal location points were plotted on the GRASS GIS to examine the extent of intermixing and illustrate animal home range estimates. Home ranges estimates were computed using a Personal Computer program developed at the University of Idaho and were examined under three different techniques; Minimum Convex Polygon (MCP), Harmonic Mean, and Bivariate Ellipse. Home ranges of 19 radio-equipped males were calculated and used for comparison purposes because all animals fit ($p \leq 0.1$) Cramer von Misses weighted bivariate distribution tests (Samuel and Garton 1985).

2.5 Habitat Use and Dietary Analyses

Methods used to analyze habitat use and seasonal diets of the Hanford Site herd are described in the following subsections.

2.5.1 Vegetation Cover Type Use Versus Availability

The analysis of habitat use and selection by deer was based only on those animals with a relatively large number of relocations (35 locations or more), and previous site characterizations that generated GIS vegetation community map layers (Downs et al. 1993). Habitat use (selection, avoidance, or indiscriminate) was determined by comparing the proportion of time animals were found within each of these different cover types to the amount of cover type that potentially was available to each animal. Potentially available habitat included any vegetation cover type that comprised at least 3% of an animal's 100% minimum convex polygon home range.

We divided the percentage of locations observed in any vegetation type by the percent of locations expected if the animal used all vegetation cover types within its home range indiscriminately. If the resulting value was greater than one, the data suggested an animal was selecting for that particular vegetation cover type, or conversely, could suggest avoidance of a vegetation cover type if the value was less than one. We determined 95% confidence intervals using the Bonferroni z statistic (Neu et al. 1974) for each animal within each available cover type. Lastly, the radio-equipped animals ($n = 15$) were tallied by vegetation cover type categories available to them and the use/availability test results (selection, avoidance, indiscriminate) (see Appendix C).

2.5.2 Deer Diets

Seasonal diets were determined for several radio-equipped male deer residing in the southern and northern study areas on the Hanford Site throughout a 1-year period. Beginning in February 1994, radio-equipped bucks from each region were selected randomly each month and observed until the animal defecated. Observers then located and collected the fecal pellet group, labeled the sample bag with the unique animal radio-frequency, date of collection, and location. Samples were stored in a freezer submitted to the Washington State University (WSU) Diagnostic Laboratories (Wildlife Habitat Lab, Washington State University, Pullman, Washington) for analysis. Preliminary efforts resulted in an average of one fecal sample per day, and indicated a maximum of 20 samples could be collected per month.

Fecal analysis is a micro-histological technique involving the determination of food habits by identifying and quantifying discernible food fragments in animal fecal material as described by Korfhage (1974) and Davitt (1979). Fecal material collected in the field was gently broken down by several minutes of agitation in a household-type blender. The fecal material was then washed through a 200-mesh screen to remove foreign material (e.g., dirt and non-discernible food fragments), stained, and mounted on microscope slides. Plant tissue collections of the local flora processed in a similar fashion were used as reference vouchers. Plant epidermal tissues were studied for particular cell morphological structures that are useful for identifying the plant species. These identifying features included: 1) the presence, morphology, and density of trichomes; 2) size and shape of individual cells; 3) stomata size, arrangement, and abundance; and 4) the presence of specialized cells and crystals.

For each sample, the proportion of area covered by plant cuticle and epidermal fragments was quantified for 25 randomly located microscope views on each of eight slides (a total of 200 views). A 10 square x 10 square grid mounted in the eyepiece of the microscope was used to measure area covered by each positively identified fragment observed at 100x magnification. Measurements of area covered were recorded by plant species (any species greater than 5% of diet), genus (less than 5%), and forage class (all grasses, forbs, and shrubs). Percent diet composition was calculated by dividing the total cover of each plant by total cover observed for all species, then multiplied by 100.

Fecal samples from different animals were composited by region of use (south vs. north) and by season. Three seasons (fall, spring, and summer) were chosen based on plant community changes typically seen in the shrub-steppe regions of south-central Washington: Fall - Sept, Oct, Nov, Dec; Spring - Jan, Feb, Mar, Apr; and Summer - May, Jun, Jul, Aug. Three duplicate composite samples were submitted for each region and for each season. Eighteen samples were submitted to the WSU Diagnostics Lab.

2.6 Testicular Atrophy: Specific Observations

This section describes materials and methods use to evaluate adult male deer found on the Hanford Site with atypical, velvet-covered antlers. We examined the frequency and extent of males on the Site exhibiting this anomaly and compared the ages and movements of the affected male deer with normal male deer, as described below.

2.6.1 Frequency and Extent of Anomaly

Because permanently velvet-covered antlers were characteristic of males with testicular atrophy, we examined frequencies of affected to unaffected males when antlers are normally calcified. We observed deer during random capture events, while tracking radio-transmitted males, and while conducting systematic roadside observations. Initial estimates of the proportion of affected animals were obtained in February and March 1991 and 1992 from captured individuals. Routine ground radio-tracking of several male deer provided an estimate of the frequency of affected males for fall and winter 1992 and 1993. For long-term monitoring, we conducted 10 roadside surveys from August through December 1994 along approximately 60 km of road beginning in the southern area and ending in the northern area. Bucks were visually examined and antlers were determined to be calcified or velvet covered. Roadside surveys also were conducted on ALE.

2.6.2 Age Distribution: Affected Versus Normal Deer

Age data (see sex and age classification methods) were grouped, reflecting the ages of each animal at the time of initial capture. Because capture efforts were biased toward affected animals, the proportion of animals that exhibited permanently velvet-covered antlers was adjusted to reflect the mean proportion of affected animals observed during 1994 road surveys.

2.6.3 Movements: Affected Versus Normal Deer

We used radiotelemetry to monitor movements of affected and normal bucks on the Hanford Site in the northern and southern study regions. Using this tool we were examined the spatial relationships of deer to known contaminated areas on the Site, as well as to natural events (wildfires), which have been postulated as being a primary causative agent of deer testicular atrophy (DeMartini and Connolly 1972).

2.7 Microscopic and Physiologic Sampling and Analyses

During 1993 and 1994, we collected blood from the jugular vein of captured deer and placed samples in glass tubes. Blood samples were stored on ice in a cooler until that evening when sera and blood constituents were separated by centrifugation and immediately frozen at -20°C.

We subsequently sedated animals with 3 to 5 mL of a 1 to 3 mixture of xylazine-HCL (Rompun - 100 mg/mL, Miles Inc., Shawnee Mission, Kansas) and ketamine-HCL (Ketaset - 100 mg/mL, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) intramuscularly and surgically removed one or both testicles from 12 affected bucks. We also removed the left testicle from six apparently normal males. Immobilized animals were given 5 mL of yohimbine-HCL intravenously (5 mg/mL - Antagonil, Wildlife Lab. Inc., Fort Collins, Colorado) to reverse the actions of xylazine-HCL and subsequently were released near the capture site (Mech et al. 1985; Kreeger et al. 1986).

2.7.1 Selected Tissue Weights/Histology

Gross and microscopic examinations were made of the testes. Testes were measured, photographed, and weighed before and after removal of the epididymis, vascular plexes, and tunics. On occasion,

radio-equipped bucks were reported to be hit on a roadway and some were sacrificed in 1995 and 1996. For these bucks, samples of brain, hypothalamus, pituitary, thyroid, lymph node, heart, lung, liver, adrenal gland, and spleen were collected and prepared for microscopic examination. Specimens were fixed in 10% formalin, paraffin embedded, sectioned at 5.0 μm, and stained with hematoxylin and eosin. We stained sections of one testicle with periodic-acid schiff (PAS), trichome, Prussian blue, and Congo red (Thompson 1966).

2.7.2 Infectious Diseases

Sera were tested by the University of Georgia (Southeast Cooperative Wildlife Disease Study, College of Veterinary Medicine, Athens, Georgia) for antibodies against epizootic hemorrhagic disease virus (EHDV) and bluetongue virus by agar gel immunodifusion (AGID), and serum neutralization (Stallknecht et al. 1995). Sera were tested for *Brucella* spp. antibodies using the standard buffered acidic plate antigen test (MacMillan 1990) by the Washington State Department of Agriculture (Olympia, Washington).

2.7.3 Sera: Hormones and Basic Constituents

Levels of follicle stimulating hormone (FSH), luteininzing hormone (LH), and testosterone (T) were determined using radioimmunoassay as described by Berndtson et al. (1974) and Nett et al. (1975, 1979). Serum chemistry was conducted using an automated system (Boehringer Mannheim/Hitachi 747, Boehringer Mannheim Corp., Diagnostics Lab System Division, Indianapolis, Indiana) by Phoenix Central Laboratory (Everett, Washington). Thyroxine (T_4) levels were determined using monoclonal solid phase radioimmunoassay (Becton-Dickinson, Mississauga, Ontario, Canada).

2.7.4 Liver Induction Enzymes

For this study, livers collected from both affected and normal deer were analyzed by the preparation of cytosol and microsomes and determination of the total cytochrome P450 (CYP) specific content, the levels of CYP1A1 by immunoblotting and the catalytic activity of glutathione S-transferase (GST). Eleven bucks (six affected and five normal) were sacrificed in 1995 and 1996, and approximately 10 g of liver was immediately collected, labeled, and placed on dry ice in a field cooler. The samples were then sent 1-day express mail to Oregon State University's Biomedical Sciences Center (Wigand Hall, Corvallis, Oregon) for enzyme analyses as described below.

Microsomal preparation. Microsomes were prepared using the following procedure (Guengerich 1989). Livers were homogenized in ice-cold homogenization buffer (10 mM potassium phosphate, pH 7.5, containing 0.15 M KCl, 1 mM EDTA and 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and the homogenate centrifuged at 10,000 g for 20 min (at 4°C) to remove cell debris, nuclei, and mitochondria. The supernatant was centrifuged at 100,000 g for 90 min to obtain the microsomes. The supernatant (cytosol) was saved and frozen (-80°C). The microsomal pellet was resuspended in 0.1 M potassium pyrophosphate, pH 7.4, containing 1 mM EDTA and 0.1 mM PMSF and recentrifuged at 100,000 g for 90 min. The washed microsomal pellet was resuspended in microsomal storage buffer (0.1 M potassium

phosphate, pH 7.25, containing 30% glycerol, 1 mM EDTA and 0.1 mM PMSF) and stored at -80°C. The protein concentration in the microsomes was determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Determination of total CYP levels in deer microsomes. Total P450 levels were measured spectrophotometrically by a modified procedure of Omura and Sato (1964): The microsomal samples were diluted in storage buffer (1:10) and saturated with carbon monoxide (bubbling gently for approximately 1 min). Afterward, the sample was divided in two equal fractions and placed into cuvettes, which were balanced in a Cary-219 spectrophotometer (automatic baseline correction mode). Next, sodium dithionite ($Na_2S_2O_4$) was added to the sample cuvette, and the difference spectrum was obtained. The concentration of CYP is then determined as follows:

$$[(A_{450\text{-}490})\text{observed} - (A_{450\text{-}490})\text{baseline}]/0.091 = \text{nmol cytochrome P-450 mL}^{-1}.$$

The specific content is the CYP concentration divided by the protein concentration and is in units of nmol/mg protein.

Analysis of CYP1A1 protein levels. The microsomal proteins were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 36 µg protein loaded per sample) by the method of Laemmli (1970) and electrophoretically transferred onto nitrocellulose membranes (Towbin et al. 1979). The nitrocellulose sheets containing the deer microsomal proteins are probed with antibody to rat CYP1A1 and the CYP1A1-antibody complex visualized by chemiluminescence using the ECL system from Amersham.

Analysis of Glutathione S-transferase (GST) activity. GST activity in the deer liver cytosol was measured by monitoring the changes in absorbance at 340 nm on a Cary-219 spectrophotometer using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (Habig et al. 1974). The reaction mixture was composed of 0.8 mL 125 mM potassium phosphate buffer, pH 6.5 (prewarmed to 25°C), containing 0.07 mL reduced glutathione (GSH) in 12.5 mM potassium phosphate, pH 6.5 (on ice), 0.02 mL substrate (in DMSO), and 0.09 mL H_20 (25°C). The reaction was initiated by the addition of enzyme (cytosol, kept on ice), which was diluted with homogenization buffer 50-100 times before using. A good linear working range was used for calculations. One unit is defined as that amount of enzyme that will catalyze the formation of 1 µmole of product per minute under the conditions used. The specific activity is defined as the units of enzyme activity per milligram protein as measured by Lowry et al. (1951).

2.8 Contaminant Levels in Deer

Tissues from several deer were sampled throughout this study and analyzed by the Battelle Marine Sciences Laboratory (Sequim, Washington) for selected organic and inorganic chemicals suspected or known to exist in various ecological compartments (i.e., ground water, soils, vegetation) on the Hanford Site. These analyses were related to the testicular condition (microscopic determination) of the animal at the time of capture and their subsequent movements as determined by radio-telemetry. Levels of these chemicals found in the Hanford Site deer were compared with levels found in deer collected from Boardman, Oregon.

2.8.1 Sample Collection Procedures

All tissue samples used for chemical analyses were collected in the field using a stainless steel knife washed with deionized water and alconox detergent between each sampling event. Sterile surgical gloves were worn and all samples were briefly rinsed with water and placed in Eagle-Picher (Miami, Oklahoma) amber glass containers with QA level 1 inorganic and organic certifications and placed in coolers until that evening.

2.8.2 Sample Analysis Procedures

Chemical (nonradiological) analyses of samples were conducted at the Marine Sciences Laboratory. Procedures are described below for organics, metals, and radiologicals. Details are included in the appendices.

Metals. Tissue samples were digested according to a modified version of EPA Method 200.3 which includes heating the samples with nitric acid and hydrogen peroxide. Digestates were then analyzed by either U.S. Environmental Protection Agency (EPA) method 200.8, inductively coupled plasma mass spectrometry (ICP -MS) or by EPA method 200.9, graphite furnace atomic absorption (GFAA).

Organics. Analysis of samples required a 10- to 20-g (wet wt.) aliquot of tissue extracted with methylene chloride using the roller technique under ambient conditions following a procedure based on methods used by the National Oceanic and Atmospheric Administration for their Status and Trends program (Krahn et al. 1988). Samples were then cleaned using silica/alumina (5% deactivated) chromatography followed by HPLC cleanup (Krahn et al. 1988). Extracts were analyzed for polychlorinated biphenyls (PCBs) and chlorinated pesticides using Gas Chromatography/Electron Capture Detection (GC/ECD), following a procedure based on EPA method 8080 (EPA 1986). The column used was a J&W DB-17 and the confirmatory column was a DB-1701, both capillary columns (30 m x 0.25 mm I.D.) All chlorinated compound results were confirmed using a second dissimilar column. Results for each column must be within a factor of two of each other to be considered a confirmed value.

Radiologicals. Procedures used to sample tissues for radiological analyses were identical to those described for metals and organics. Radiological analyses of samples were conducted by Quanterra Environmental Services. The laboratory participates in DOE's Quality Assessment Program and EPA's Laboratory Intercomparison Studies. For a complete review of Quanterra's analytical methods see Hanf and Dirkes (1995).

3.0 Results and Discussion

This section summarizes results of our 1993-1996 field and laboratory work related to the study objectives outlined in Section 1.0. Supporting data for these studies are included in Appendices A-F.

3.1 Population Characteristics

The following subsections report results of field studies on Hanford Site mule deer conducted as part of our objectives to estimate population composition, determine natural and human-induced causes of mortality, and examine the reproductive status of the herd in support of our examination of testicular atrophy. See Appendices A-F.

3.1.1 Sex and Age Structure

Table 3.1 shows the age and sex ratios of Hanford's Columbia River deer populations. Data collected from roadside surveys during the pre- and post-hunting periods from 1993 through 1996 suggested that a relatively high proportion of bucks occur onsite.

The buck:doe:fawn ratios determined from roadside surveys conducted from July through August 1993-1995 averaged 205 deer observations per year. Standardized to 100 does, an average of 47 males (± 2.9 1 S.E.) and 26 fawns (± 3.8 1 S.E.) was found along the Columbia River (see Figure 1.1). The proportion of males estimated within these populations during the 1996 season was consistent with previous years. In comparison, data collected during a deer telemetry study in 1994 on the Yakima

Table 3.1. Deer Classifications on the Hanford Site

Pre-Hunting (July-Aug)					
Year	Bucks	Does	Fawns	# Trials	# Animals
1993	51	100	28	5	77
1994	47	100	19	10	410
1995	41	100	32	6	130
1996	51	100	9	10	173
Post-Hunting (Dec - Jan)					
Year	Bucks	Does	Fawns	# Trials	# Animals
1993	18	100	44	5	185
1994	29	100	27	9	592
1995	27	100	29	9	238
1996	24	100	17	7	252

Training Center (YTC) demonstrated an average of 12.6 bucks per 100 does during the pre-hunting season and 7.5 bucks per 100 does after the fall hunting season (Raedeke 1995).

3.1.2 Fawn Production

Results from radio-tracking surveys of 10 radio-equipped does followed from early July through September 1991 and 20 in 1992 to monitor postnatal survival of their fawns (Appendix B) showed that only one of six does observed with fawns in July actually lost its fawn by September. These data suggest that fawn mortalities on the Hanford Site mostly occurred either neonatally or immediately after birth. In addition, because some radio-equipped does were found with fawns only after July, we believe post-hunting fawn ratios to be the most accurate indication of recruitment.

Data shown in Table 3.1 suggest no significant differences ($p \geq 0.4$) in the yearly pre-hunt and post-hunt fawn-to-doe ratios on Hanford. Fawn-to-doe ratios observed on the Hanford Site in previous years seem to complement observations on the YTC as 26 fawns per 100 does were observed during a helicopter survey ($n = 156$) in November 1994 (see Raedeke 1995). A relatively lower number of fawns was observed on the Hanford Site during the 1996 pre-hunting survey. However, the post-hunting survey results suggested no statistically different fawn ratios when compared with 1994 and 1995 (see Table 3.1). The 1996 results were statistically lower ($p \leq 0.1$) than the fawning ratio observed in 1993. In addition, Table 3.1 depicts a general downward trend in the post-hunt fawn to doe ratios. Both the YTC and Hanford Site fawn-to-doe ratios appear relatively low compared to most mule deer populations recently surveyed in Oregon where an average of 58 fawns per 100 does was determined (ODFW 1995).

The reproductive capacities of deer populations (i.e., number of fawns successfully recruited into the adult population) are highly variable and largely depend on habitat and forage conditions (Taber 1953; Taber and Dasmann 1957; Wallmo 1981). In addition, the reproductive capacity of any deer herd may be affected when bucks are in such limited supply that mature does forego copulation, and hence, recruitment of fawns in the subsequent year. Selective harvests of male deer by hunters may reduce the ratio of males to females such that not all females become fertilized each year. Roadside surveys demonstrated a relatively high proportion of male deer in the Hanford herds. Even considering that approximately 25% of these animals are reproductively inactive, the pregnancy rates found in several females suggest this is not a limiting factor.

3.1.3 Pregnancy Rates

Blood tests for pregnancy specific protein B (PSPB) for the 13 adult female deer captured in 1993 indicated that all does (100%) were pregnant, also suggesting that a sufficient number of fertile males were present in the population to maintain reproductive capacity of the herd. This blood test indicated only if the female is pregnant. It did not indicate the number of fetuses present.

3.1.4 Adult Mortalities

Of the 20 male deer residing near the Columbia River radio-tagged in 1994, four (19%) of the males were harvested during the 1994 hunting season, three (14%) legally and one (5%) illegally on the Saddle Mountain Wildlife Refuge near State Highway 24.

Deer hunting is common on lands adjacent to the Hanford Site, and in particular, on the west side of Rattlesnake Mountain (see Figure 1.1). Until recently, however, little information was available regarding hunting pressure on riverine islands and along the shorelines of the Hanford Reach. Eberhardt et al. (1979) studied movements and mortality rates from fawns captured near the Columbia River and found hunting on adjacent private lands to be the most common cause of mortality, typically for animals 1.5 years old.

Based on data collected from radio-equipped deer from 1991 to 1994, an average of 11% (range 0 to 37% at the 95% confidence level) of the male deer residing along the Columbia River may be harvested in one year. Natural causes of mortality were minimal (less than 1% of total moralities). Deer harvest results in this study are consistent with Eberhardt et al. (1982) as they estimated the probability of a female deer being legally or illegally harvested during any given year at 8% (range 0 to 21% at the 95% confidence interval).

Anderson et al. (1974) simulated a selective harvest of male deer and showed that an annual kill of 25% of bucks (two point or better) would result in an average ratio of 18 bucks per 100 does in the post-hunt population. With no hunting, the buck-to-doe ratio was 48 bucks per 100 does.

3.1.5 Age-Specific Survival

A survival table describes the pattern of deaths (or survival) by age-class and provides some insight into whether certain age-classes within the population are surviving as expected (Bookhout 1994). Survivorship rates for *Cervids* (deer) typically take a Type I curve, low mortality early in life (not including fawn losses), and higher rates among older individuals (Wallmo 1981). Table 3.2 presents age-specific survival estimates for radio-equipped male deer monitored on the Hanford Site from 1993 through 1995. Although our sample sizes per each age-class (each year of life) are relatively low, the grouped averages are not, and indicate typical Type I suvivorship rates.

Table 3.2. Age-Specific Survival Rates for Male Deer on the Hanford Site

	AGE CLASSES (years)													
YEAR	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14
1993	0 (2)[a]	1 (11)	0 (2)	0 (1)	0 (2)	1 (2)	0 (0)	0 (3)	0 (2)	0 (0)	0 (2)	0 (1)	0 (0)	0 (0)
1994		1 (2)	2 (10)	0 (2)	0 (1)	0 (2)	0 (1)	0 (0)	0 (3)	0 (2)	0 (0)	0 (2)	1 (1)	0 (0)
1995			0 (1)	0 (8)	0 (2)	0 (1)	0 (2)	0 (1)	0 (0)	0 (3)	0 (2)	0 (0)	0 (2)	0 (0)
% SURVIVAL RATES PER AGE CLASS														
	100%	85%	85%	100%	100%	80%	100%	100%	100%	100%	100%	100%	67%	-
GROUPED AVERAGES	**AGES 0 to 2**			**AGES 3 to 5**			**AGES 6 to 8**			**AGES 9 to 11**			**AGES 12 +**	
	86%			**95%**			**100%**			**100%**			**67%**	
# SAMPLES	28			21			12			12			3	

[a] 0 (2) = 0 MORTALITIES FROM A SAMPLE SIZE OF 2

3.1.6 Age Distribution

Figure 3.1 shows the age distributions of male deer on the Hanford Site for 1993 and 1994. The age distribution of a population is simply the numbers of individuals within each age-class in the population at any given point in time (Bookhout 1994; Wallmo 1981). This information is imperative to accurately compare characteristics of one population to others. Age data collected during the 1993 and 1994 deer captures (n = 29) were grouped, reflecting the age of each animal at the time of initial capture (Appendix D) to examine the "snap shot" age distribution of male deer on the Hanford Site.

A relatively large proportion (over 20%) of the Hanford mule deer herd are 5 years of age or older (Figure 3.1). These data corroborate the buck-to-doe ratios, which suggest atypically high numbers of mature bucks. They also support our estimates of age-specific survivorship where survival of the young and middle age animals is high. The sharp declines in the proportion of animals living from age 3 to 4 is likely an artifact of the sample size.

In comparison, Raedeke (1995) estimated the YTC deer herd age structure to contain only 4% of male deer surviving beyond 3 years of age. Age and sex classification results collected on the YTC closely correspond to classification data from other areas where deer are heavily hunted (Musser and Stream 1994; ODFW 1995).

3.2 Population Size Estimates

The following subsections describe results of studies designed to estimate deer densities on the Hanford Site using mark-resight models, FLIR technology, and fecal pellet counts.

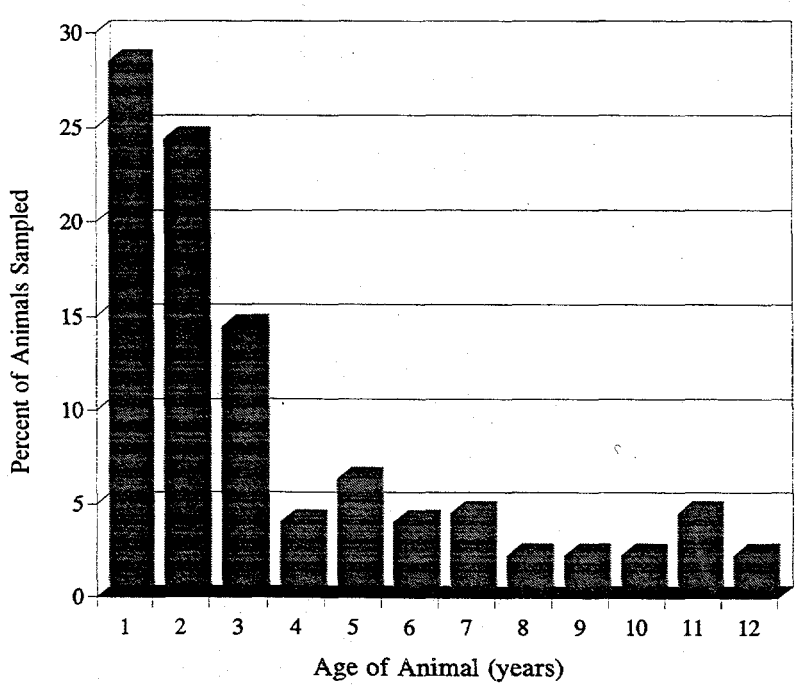


Figure 3.1. Age Distribution of Hanford Site Male Deer (1993-1994)

3.2.1 Mark-Resight

Table 3.3 shows that results of the three methods used to estimate population size were similar, except for the 95% confidence intervals of the Bowden method. The Minta-Mangel model produced the most narrow 95% confidence intervals, and the resulting density (study area 200 km^2) calculation was used to infer relative deer densities on ALE and the inner regions of the Hanford Site. Values derived from these numbers indicated the average deer density within the river region of the Hanford Site was 1.6 deer/km^2, ranging from 1.3 deer/km^2 to 2.1 deer/km^2 at the 95% confidence levels.

3.2.2 Forward Looking Infrared Technology

Deer densities in the river region estimated using FLIR demonstrated 100% detection of deer from dawn (0615 hr) to 0800 hr (Table 3.4). By 0830H, however, detection essentially dropped to 20%. For this reason, we used only FLIR survey results collected during the 100% detection-rate time period. Although temperatures (2 m above ground level) above 5°C appeared to correspond to the decrease in detection of deer using the FLIR from within the test plots, this variable is probably not a good indicator of acceptable FLIR survey conditions.

Results closely corresponded to the mark-resight population density estimates as 1.4 deer/km^2 and 1.8 deer/km^2 were found using FLIR for the two plots flown along the Columbia River (Figure 3.2; Appendix B).

3.2.3 Fecal Plots

Figure 3.2 shows the proportion of deer pellet groups found in each study region in October 1994 and 1995. The relative number (density) of mule deer fecal pellet groups found within different regions can serve as an index to the relative numbers of deer found within the region (Neff 1968; Bookhout 1994).

We found significant ($p \leq 0.01$) differences when pellet densities were grouped by region and by season. Data collected in October 1994 and 1995 demonstrated the highest proportion (significant) of pellet groups in the river and ALE (west) regions, and much lower proportions in the inner Hanford Site and ALE (east) regions. We found significant differences between the highest and lowest regions, but no difference was seen among years. Data collected in April 1995 were not significantly different among regions but were significantly higher than counts performed the following October (see Appendix B).

Table 3.3. Comparison of Population Size Estimation Techniques for River Region

Model	Population Estimate	95% Confidence Intervals
JHE	201	163-256
Bowden	182	102-327
Minta-Mangel	190	160-250

Table 3.4. Forward Looking Infrared Test Plot Results

Plot #	Time	Mean[a] Ambient (2.0 m AGL) Temperature °C	# Deer Seen by FLIR	# Deer Found by Ground Observers	Detection of Deer by FLIR (%)
J-3	0615	-0.28	3	3	100
E-13	0630	-0.88	1	1	100
D-13	0710	1.94	3	3	100
E-4	0730	3.27	6	6	100
J-3	0830	6.77	1	5	20

(a) From weather monitoring station near 100-F Area (see "edna station" in Dirkes and Hanf 1995).

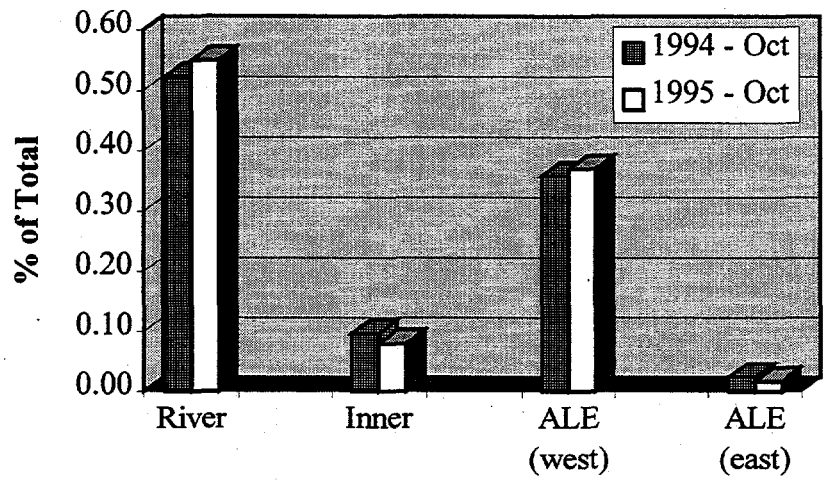


Figure 3.2. Proportion of Deer Pellet Groups Found in Each Study Region (October 1994, 1995)

Possible explanations for this apparent difference includes seasonal selection or avoidance in the deer's use of vegetation cover types and/or different digestive and defection rates as dictated by seasonal changes in forage quality. Further examination of the data is warranted, especially with respect to seasonal differences.

The actual density of pellet groups collected in 1994 is not comparable to counts conducted in 1995 as old pellet groups within each plot were not cleared in 1994 (see Appendix B). The proportion of pellet groups observed in each region in October 1995 (Figure 3.2) appeared to be similar to the proportion found on the initial check date.

We can grossly estimate the total number of deer on Hanford if we assume the following: 1) the relative density of deer pellet groups found in each region is indicative of the number of animals found

there, 2) we use average buck-doe-fawn ratios; and 3) we use the mark-resight estimated average number of deer (330 animals) in the river region. Deer abundance on ALE (west), ALE (east), and the inner regions was 70% (230 animals), 20% (66 animals), and 5% (17 animals), respectively. Approximately 650 deer reside on Hanford, not including those living across (north) the Columbia River. The low abundance of deer or deer pellets found in the inner and ALE (east) regions is reasonable, as virtually no water sources are available to deer there. However, we recommend more plots sites be established in each region and in cover types indiscriminately used by deer because sample sizes were rather small for these areas.

3.3 Deer Movements and Home Ranges

Weighted bivariate ellipse estimates for male deer on the Hanford Site suggested an average home range size of 52.3 $km^2 \pm 22.8$ km^2 (1 S.D) (Figure 3.3). These results are consistent with findings of Eberhardt et al. (1982), who reported an average home-range size of 37 deer to be 39 ± 27 km^2 (1 S.D.) using the elliptical technique.

Animals caught in the southern region of the study area ranged downriver extensively but rarely were present at any distance upriver from this location. Animals captured from the northern region (near old reactor sites) essentially were confined to these areas. From 1992 to 1994, 866 location points were collected for these deer with an average of 47 ± 9 (1 S.D.) locations per animal and were tracked an average of 17 ± 6 (1 S.D.) months.

Figure 3.3 also identifies those animals exhibiting testicular atrophy. Although essentially two subpopulations exist, affected males were found in both herds.

3.4 Habitat Use and Dietary Analysis

The following subsections describe results of our 1993-1996 field studies designed to determine Hanford Site deer habitat uses and dietary habits. Supporting data are included in Appendix C.

3.4.1 Vegetation Cover Type Use Versus Availability

Figure 3.4 generally shows the amount of each vegetation type potentially available to radio-equipped deer and the vegetation types they actually used during this study (any cover type greater than 3% of the total cover within the animal's 100% MCP home range estimation).

Table 3.5 summarizes results of habitat use versus availability analyses. The quantitative summary for each cover type is provided in Appendix C. A preponderance of animals avoided buildings and parking lots, the White Buffs, riverine wetlands, cheatgrass/Sandberg's bluegrass, and rabbitbrush/ bunchgrass cover types. Three deer which contained basalt outcrops comprising on average 10% (range 6.2 to 14.6%) of the cover within their home range, also avoided this cover type, and two of the three significantly ($p \leq 0.05$) avoided it. Six (75%) of 8 animals preferred big sagebrush/bunchgrass/ cheatgrass and riparian cover types. Nine (82%) of 11 animals selected for sand dunes/bitterbrush/ bunchgrass. Other cover types that were available to a number of animals but were neither selected for

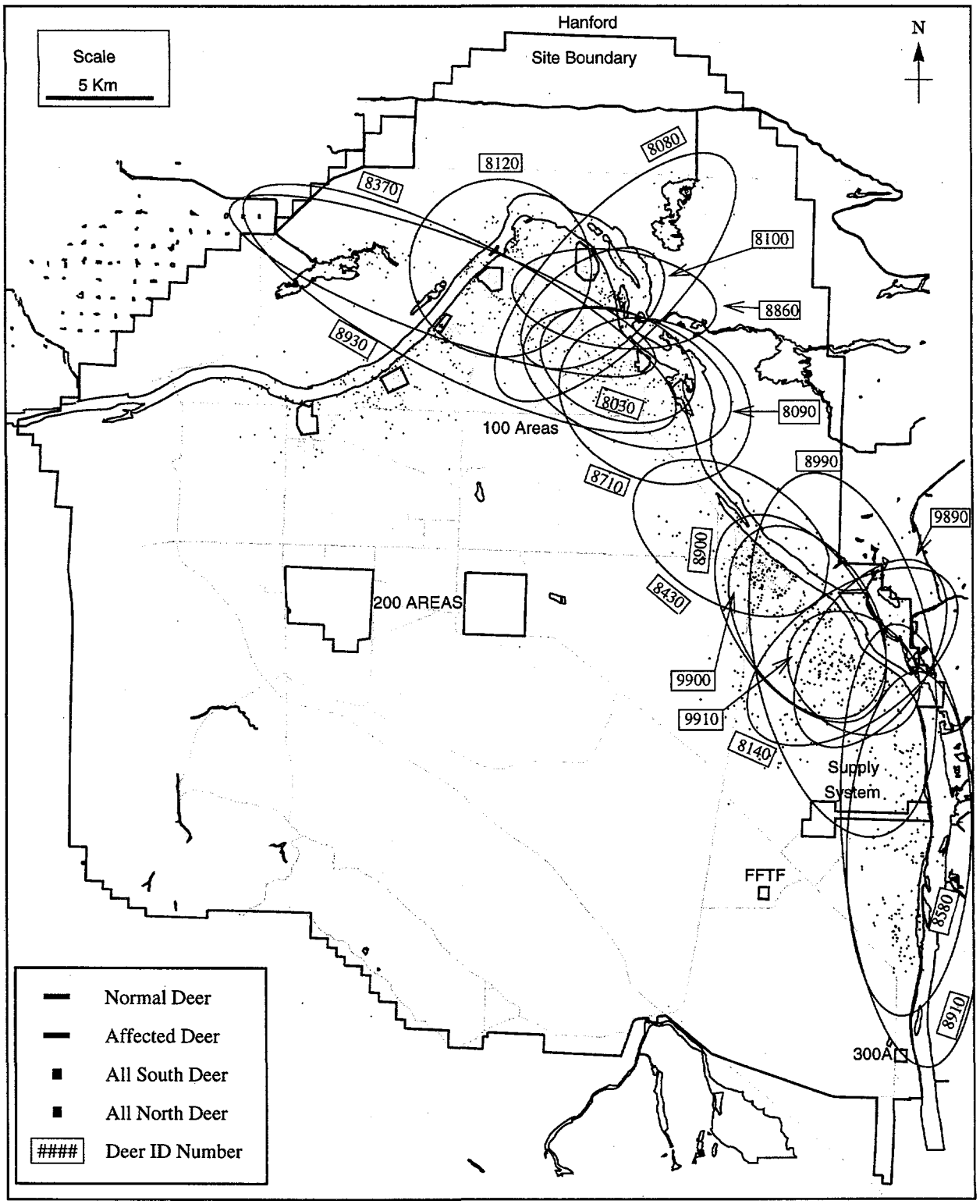


Figure 3.3. 95% Weighted Bivariate Home Range Estimates from Affected and Normal Radio-Transmittered Deer on the Hanford Site. Animal locational points are plotted and separated by north and south.

Table 3.5. Summary of Vegetation Cover Type Use Versus Availability by Radio-Transmittered Bucks on the Hanford Site

Avoidance	Selection	Indiscriminate
Rabbitbrush/bunchgrasses*	Sagebrush/bunchgrass/cheatgrass	Sagebrush-hopsage/bunchgrass/cheatgrass
Cheatgrass/Sandberg's bluegrass*	Sand dunes/bitterbrush/bunchgrass	Abandoned old fields
Buildings, parking lots*	Riparian	Post-fire shrub-steppe
White Bluffs*		
Basalt outcrops*		
Riverine wetlands*		

* Denotes significant ($P < 0.05$) results.

nor avoided included sagebrush-hopsage/bunchgrass/cheatgrass, abandoned old fields, and post-fire shrub-steppe. No statistically significant ($p \leq 0.05$) results were obtained for cover types selected for by deer. No differences between affected and normal animals were found.

3.4.2 Deer Diets

Fecal examinations of radio-equipped male deer indicated they frequently consumed woody species (shrubs) growing along the shoreline (Appendix C). Six composite dietary analysis results from both the northern (n = 11 deer) and southern (n = 11 deer) river regions collected during the summer season 1994 (May - August), showed that shrubs comprised nearly 70% (range 60.3 to 75.9%) of the animal's diets (Figure 3.5). Of the shrub species identified in fecal samples, willow (*Salix spp.*) and mulberry (*Morus alba*) were common and are only found along the riparian zone of the Columbia River. Samples analyzed from the southern region deer also contained a large portion of shrubs; however, the predominant shrub was bitterbrush (*Purshia tridentata*), an upland shrub, thus reflecting the availability of this palatable browse species there.

Spring season diets (Jan - April) suggested foraging preference for succulent grasses as opposed to shrubs (Appendix C). In both study regions, spring season results (n = 19) indicated less than 7% (range 3.3 to 8.2%) of the deer's diet consisted of woody plants. The dominant grass species present in samples collected in spring were cheatgrass and Sandberg's bluegrass. Forbs also were consumed more than shrubs during the spring season and were dominated by evening primrose (*Oenethara spp.*) and lupine (*Lupinus spp.*), suggesting consumption of plants primarily occurred away from the riverine system during spring months.

The notable difference between diets of deer residing in the northern and southern study regions was found during the fall period. Shrubs, primarily mulberry and willow, on average comprised 63% (range 56.4 to 69.9%) of the deer's diet in the northern region as opposed to 23% (range 19.0 to 25.4%) in the

- Big Sagebrush-Spiny Hopsage/Bunchgrasses-Cheatgrass
- Post-Fire Shrub-Steppe on the Columbia River Plain
- Riverine Wetlands and Associated Deepwater Habitats
- Big Sagebrush/Bunchgrasses-Cheatgrass
- Buildings/Parking Lots/Gravel Pits/Disturbed Areas
- Snow Buckwheat/Indian Ricegrass
- Rabbitbrush/Bunchgrasses
- Cheatgrass-Sandberg's Bluegrass
- Abandoned Old Fields
- Cliffs (White Bluffs)
- Basalt Outcrops
- Riparian

Figure 3.4. Vegetation Cover Types on the Hanford Site, Deer Locations (black dots on map), and 100% Minimum Convex Polygon Home Ranges of Radio-Transmittered Bucks (white polygons on map)

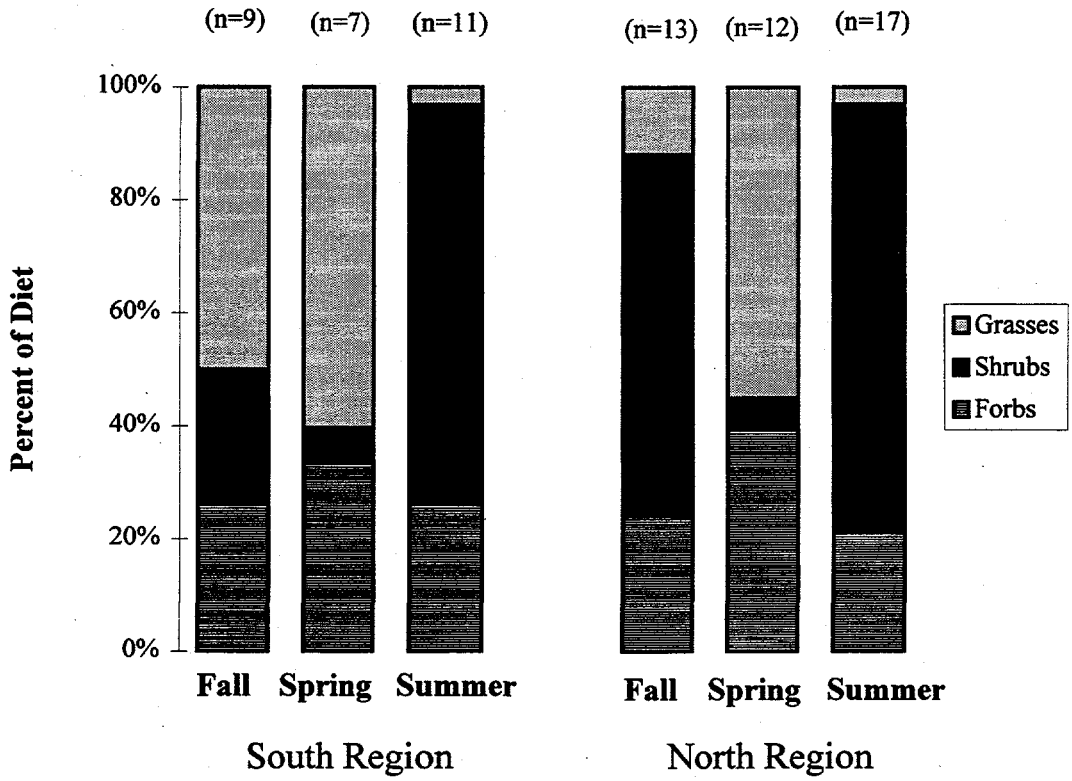


Figure 3.5. Composition of Diets from Radio-Transmittered Deer by Region and Season (n = #)

southern region Deer diets from the southern region contained over 30% Sandberg's bluegrass as opposed to only 4% in the northern region. These data again reflect the availability of late-summer succulent grasses occurring within the two regions (Downs et al. 1993). The northern region largely consists of old abandoned farm fields where cheatgrass is predominant. Cheatgrass generally becomes desiccated in early summer, whereas Sandberg's bluegrass persists as a succulent grass through September. The southern area comprises relatively undisturbed land where native species such as Sandberg's bluegrass are common.

Plants that are known to have reproductive effects by producing high quantities of estrogens and/or toxins were consumed by deer in both regions. The most commonly consumed plants fitting this description included lupine (*lupinus spp.*), loco weed (*Astragalus spp.*), and sweet clover (*melilotus spp.*) (see Appendix C).

3.5 Testicular Atrophy: Specific Observations

This section describes results of our examinations of adult male deer with atypical, velvet-covered antlers and comparisons between these animals and normal male deer on the Hanford Site. The frequency and extent of this anomaly is described along with the age distribution and movements of affected versus normal deer and changes over time.

3.5.1 Frequency and Extent of Anomaly

Four (22%) of 18 adult males captured in 1991 and 1992 had velvet-covered antlers (Figure 3.6) and lacked normally developed testicles (Figure 3.7). Based on observations while radio-tracking male deer in fall and early winter 1992 and 1993, 13 (15%) of 88 bucks exhibited atypical, velvet-covered antlers. Systematic roadside observations in 1994 indicated 23 ± 3% 1 S.E. (n = 116) of the male population was affected. Although sample sizes were low when data were separated by region, no difference in the frequency of affected animals was observed between the northern and southern study regions. None of the male deer observed during fall and winter 1994 and 1995 had velvet-covered antlers on the ALE Reserve. However, we know that at least one deer exhibiting velvet-covered antlers and small testicles was harvested from property adjacent to ALE in 1992 (D. Smasne, personal communication 1992).

Systematic roadside observations passing through the north and south study regions in 1994 on the Hanford Site closely corresponded to these previous efforts, indicating 23± 3% (1 S.E.) of the male population was reproductively impaired. Although sample sizes were low when grouped by study region (total n = 116 observations), no difference in the frequency of affected animals was observed between the northern and southern areas. In contrast, less than 1% of the male deer found on the ALE Reserve had velvet-covered antlers (L. Fitzner, WDFW, personal communication 1995).

Causes of atypical, velvet-covered antlers in mule deer have been extensively studied and were summarized by Brown (1983). Wislocki et al. (1947) described the hormonal control of the antler growth cycle, proposing that gonadal and antler cycles in deer are controlled primarily by the anterior pituitary and testes. Goss (1968) studied inhibition of growth and shedding of antlers by reduced levels of oestradiol and testosterone and supported the theory that annual cycles of antler replacement are coordinated by seasonal fluctuations in sex hormones (Wislocki et al. 1947).

The cause or causes of testicular atrophy in deer is or are presumably unknown. Cases of atrophic testes in deer *(Odocoileus* spp.) have been reported, but very few demographic or physiologic investigations have been conducted to determine the etiology of the disease (Murphy and Clugston 1970; DeMartini and Connolly 1972; Robinette et al. 1977). Clarke (1916) first documented atypical, permanently velvet-covered antlers in *Odocoileus* species of the Pacific slope. Taylor et al. (1964) documented a similar occurrence in white-tailed deer (*O. virginianus*) in Texas, but cause was not determined. They observed bucks with atrophied testes and velvet antlers (7% of adult males killed) to be more prevalent on granite-gravel soil types as compared to non-granite soil types within their study area. The researchers suspected that some toxic agent in the soil might be responsible. Clark (1953) reported that deer with abnormal antler growth and retained velvet (referred to as "cactus bucks") were present in Arizona and had testes "the size of an average marble and almost as hard." Robinette et al. (1977) also reported at least 3 (0.06 %) of 4,670 hunter-killed bucks in Utah had atrophied testes.

Several workers have investigated the occurrence and causes of testicular atrophy in domestic animals (Ladd 1985), but none have determined the cause of gonadal atrophy in deer. Possible etiologies for testicular atrophy by direct or indirect (hormonal) pathways include heat stress, brucellosis, phytoestrogens, mycotoxins, vitamin and mineral deficiencies, high doses of heavy metals, organochlorine contamination, and many others (Robbins 1983; Ladd 1985; Blanchard et al. 1991; Diekman and Green 1992; Colburn et al. 1996).

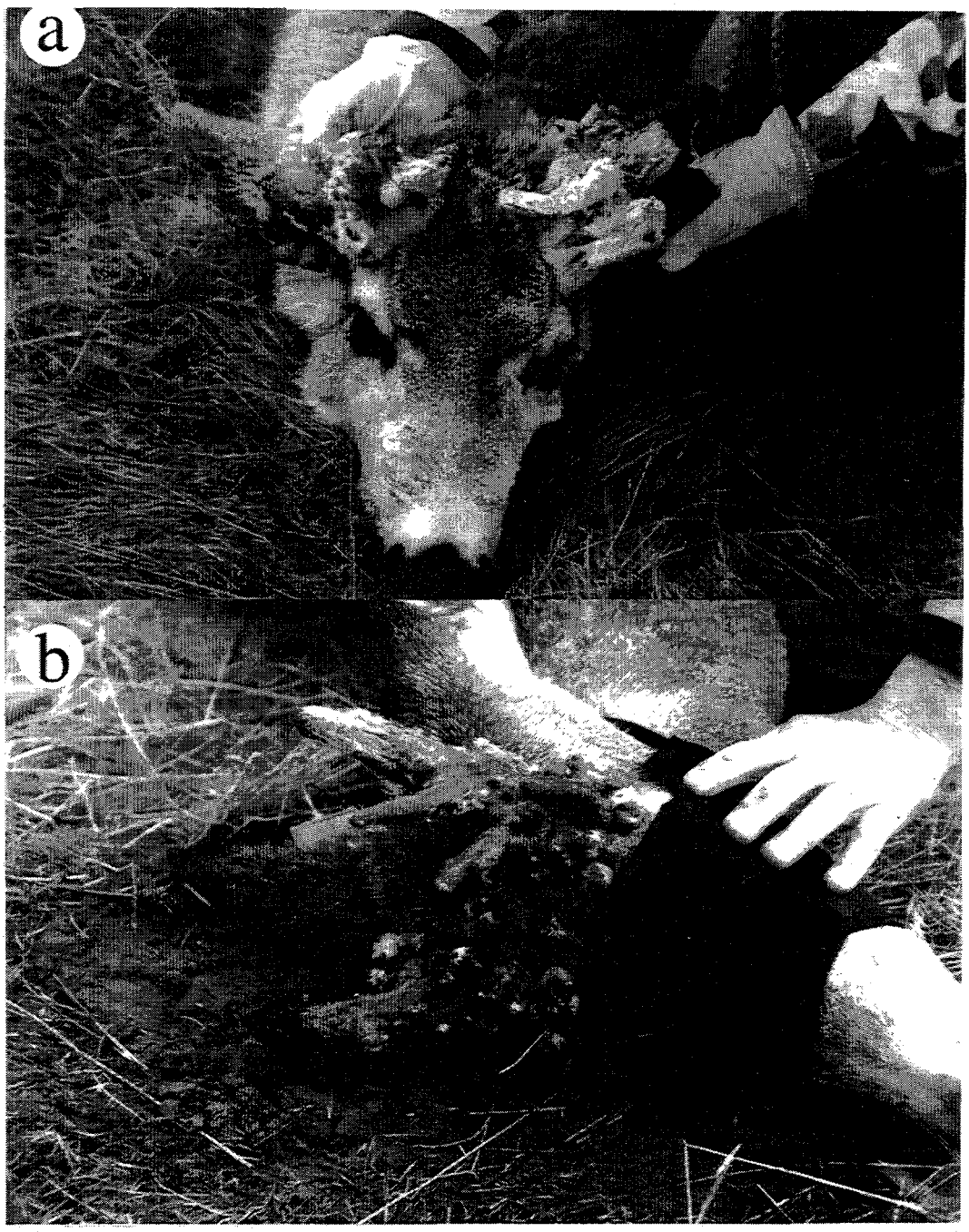


Figure 3.6. Photograph of (a) Affected Animal (Number 148.860 - 12 years old) and (b) Affected Animal (Number 149.900 - 9 years old)

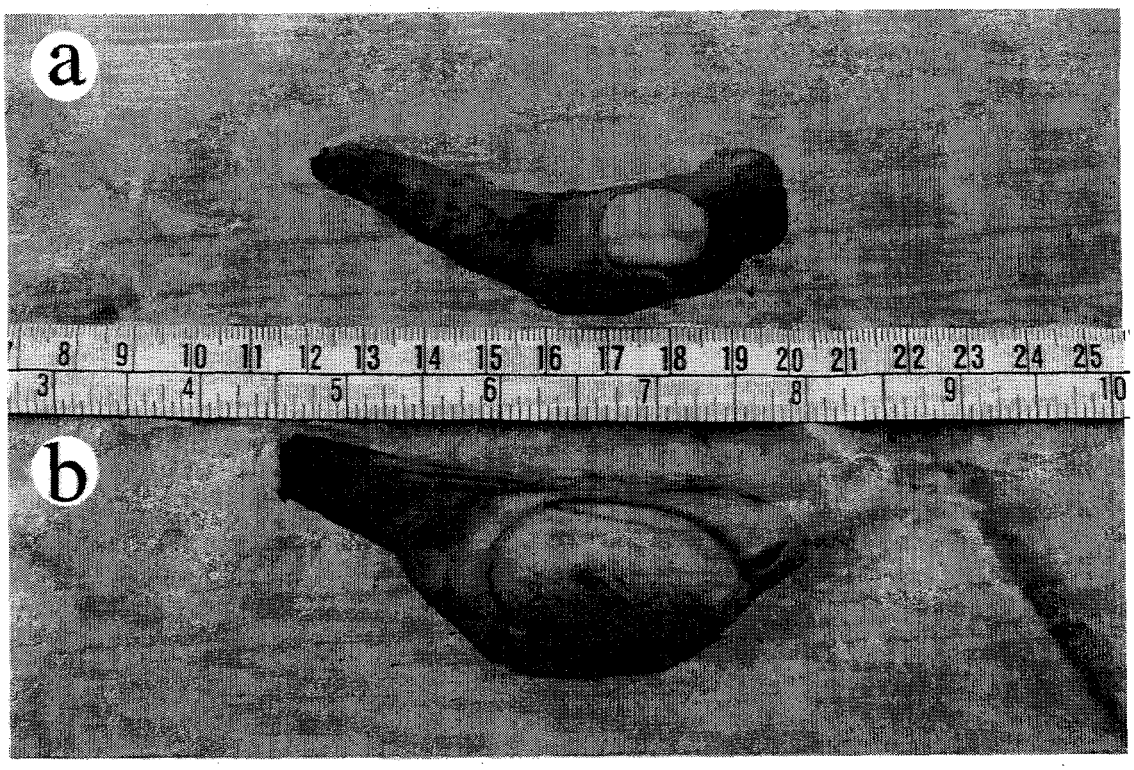


Figure 3.7. Testis from (a) Affected Animal (Number 148.250 - 10 years old) and (b) Normal Animal (Number 148.210 - 4 years old)

3.5.2 Age Distribution: Affected Versus Normal Deer

Based on the ages of 17 apparently normal males with calcified antlers and 12 males with atypical, velvet-covered antlers, only the relatively older animals (mostly >5 years of age) were affected (see Figure 3.8). In this figure, age data were grouped, reflecting the ages of each animal at the time of initial capture (see Appendix D). Because capture efforts were biased toward affected animals, the proportion of animals that exhibited permanently velvet-covered antlers was adjusted to reflect the mean proportion of affected animals observed during 1994 roadside surveys. No affected animals were observed in the 1- to 2-year age group; three (22%) of 14 of the 3- to 5-year age group were affected, and all the 7^{+}-year age groups were affected.

3.5.3 Movements: Affected Versus Normal Deer

By monitoring movements of affected and normal animals in both regions using radio-telemetry we have been able to examine the spatial relationships of these deer to known contaminated areas, as well as to natural events (wildfires), which have been postulated as being a primary causative agent of deer testicular atrophy (DeMartini and Connolly 1972). Figure 3.9 illustrates the 95% weighted bivariate ellipse home ranges of affected and normal radio-equipped bucks on the Hanford Site in relation to wildfire areas mapped on the Hanford Site from 1985-1995. As shown, deer in the southern region used post-wildfire areas. Locations collected from affected animals in the northern region, however, rarely overlapped with recently burned areas (affected animals 8860, 8100, 8370, and 8080). Home ranges for some northern animals overlapped with some of the burned areas; however, the animals were rarely located within these areas during our telemetry studies (see location points in Figure 3.9).

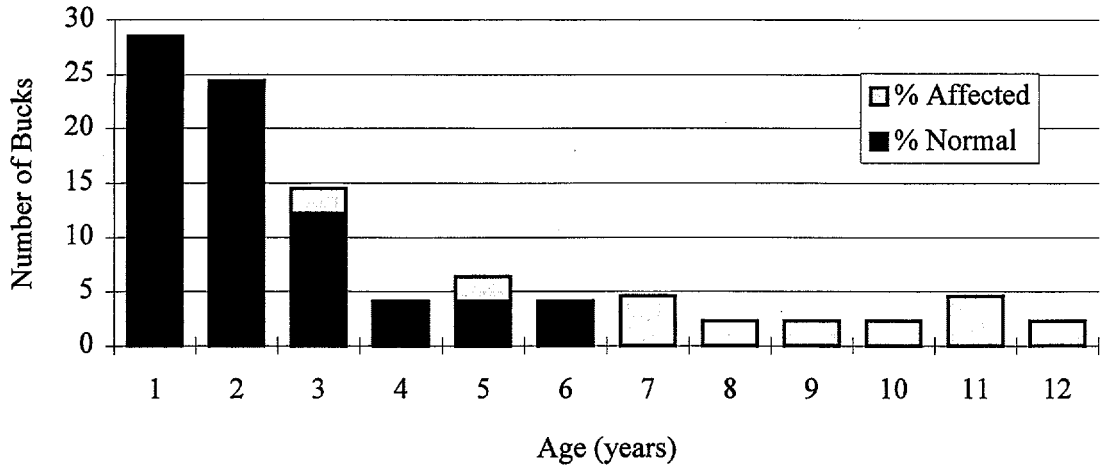


Figure 3.8. Age Distribution: Identifies Normal Hanford Site Male Deer and Those With Atypical, Velvet-Covered Antlers by Age Group

3.5.4 Changes Over Time

Field technicians closely observed most living normal radio-tagged bucks each year from September through January 1992 to 1995, when antlers are typically calcified. None of the radio-tagged animals found to be normal during capture events were observed with velvet-covered antlers during the rutting season (Appendix D). However, one previously normal male captured in 1992 was harvested on lands adjacent to the Hanford Site in fall 1995 and exhibited velvet-covered, two-point antlers. In 1993 and 1994, field technicians had observed the buck (Number 148.140) as a notably large, typical four-point. This animal spent almost the entire summer of 1995 across the river (Franklin County) in a canyon containing a spillway for water run-off from the adjacent agricultural areas (see Figure 3.8). It should also be noted that at least two normal animals found to be ages 5 and 6 in 1994 maintained typical-calcified antlers up through 1996 (see Appendix D).

3.6 Microscopic and Physiologic Analyses

The following subsections provide results of our microscopic and physiologic sampling analyses conducted on normal and affected male deer.

3.6.1 Selected Tissue Weights

Total body weights were measured during the 1994 capture events and suggested no difference between affected and normal males. The average weight measured from 11 normal males older than one year was 91 kg (range 64 to 107 kg) as compared with 88 kg (range 73 to 119 kg) for 10 affected males (Appendix D). These values are consistent with the expected range for Rocky Mountain mule deer (Anderson et al. 1974; Wallmo 1981). Fresh weights of pituitaries and thyroids were not statistically different ($p \geq 0.5$) between four animals with essentially normal testes (mean age 6.3 years) and four affected animals (mean age 9.2 years), and averaged 1.25 g (range 0.84. to 1.60 g) and 2.4 g (range 0.9

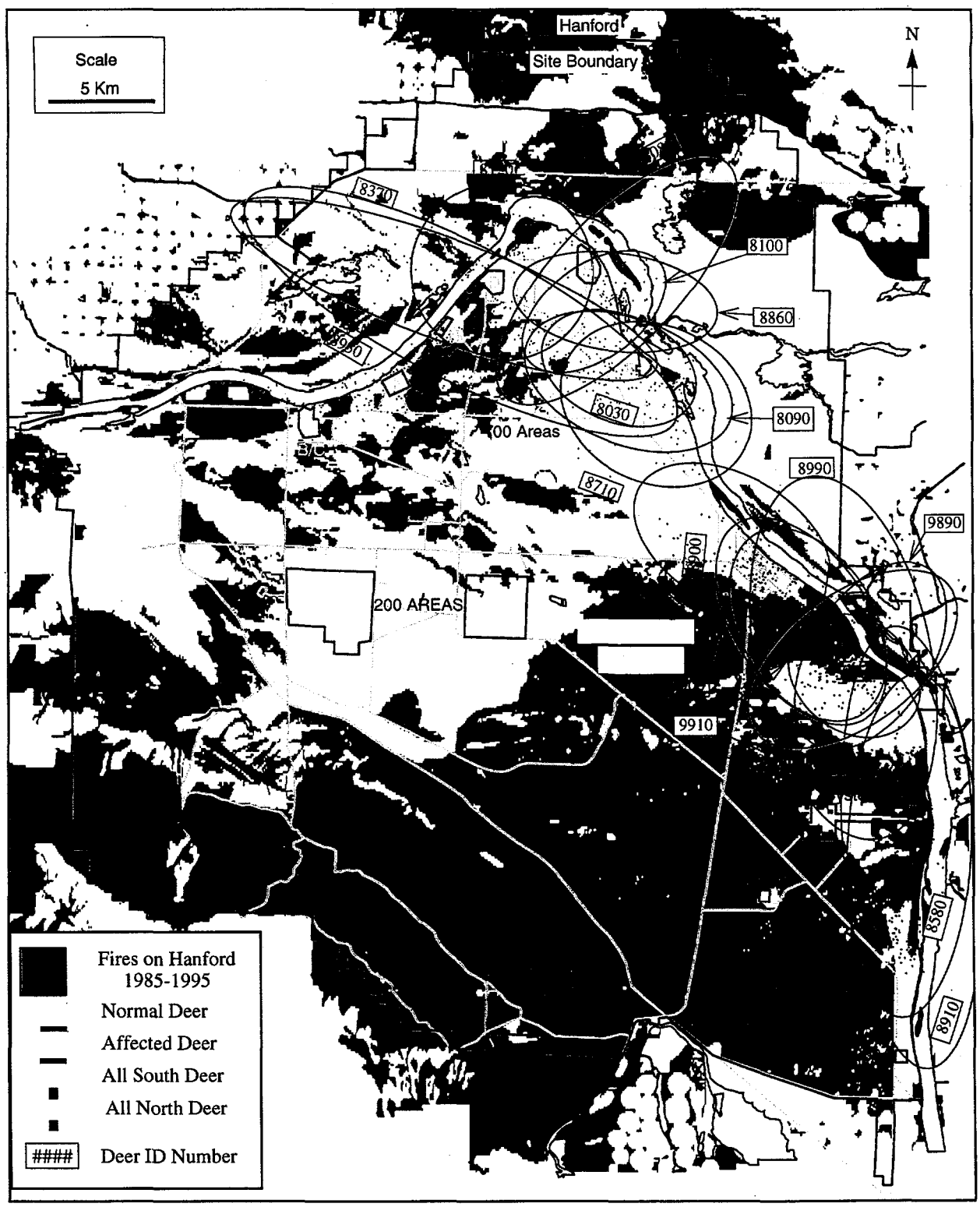


Figure 3.9. 95% Weighted Bivariate Ellipse Home Ranges and all Locations of Affected and Normal Animals in Relation to Fires on Hanford (1985 - 1995)

to 3.5 g), respectively (see Appendix D). Anderson et al. (1974) reported mean fresh weight of pituitaries and thyroids from a large sample of adult male Rocky Mountain mule deer from north-central Colorado to be 0.71 g (range 0.12 to 1.37 g) and 2.65 (range 1.17 to 6.22 g), respectively. Average thyroid weights from the Hanford deer herd are comparable to this reference; however, pituitary weights appeared to be somewhat heavier and even exceeded the reported maximum weight from a deer herd in Colorado.

Testicular weights determined in February 1994 (without plexus, epididymis, and tunics) from 11 animals with atypical, velvet-covered antlers had a mean of 4.4 g (range 0.8 to 6.5 g) compared with 10.4 g (range 6.7 to 16.1 g) for testes collected from six apparently normal animals ($p \leq 0.001$). One animal exhibiting normal antlers but slightly atrophic testes (Number 149.920) had a 20.6 g testis weight shortly after being hit by a car in March 1994. Anderson et al. (1974) determined average fresh weights of testes from adult Colorado mule deer ($n = 51$) to be 18.0 g (range 9.3 to 43.4 g.).

3.6.2 Histology

With the exception of testes, microscopic examination of lung, heart, liver, kidney, spleen, adrenal gland, pituitary, brain, thyroid, and lymph glands were essentially normal in affected deer. On histological examination of collected testes, four of the six bucks with normal antlers were essentially normal for the non-breeding season (Figure 3.10-a) These four animals were determined to be 1 year old. Testes from the other two males with normal antlers had slight tubular and leydig cell atrophy with small amounts of associated interstitial lymphocytic inflammation (Figure 3.10-b). Within areas of atrophy, and occasionally other interstitial foci, small aggregates of monocytes and histocytes were present. These animals were 3 and 4 years old.

Testes collected from 11 of the 12 males with atypical, velvet-covered antlers had marked (Figure 3.10-c) to extreme (Figure 3.10-d) tubular and leydig cell atrophy. Within this tubular and leydig cell atrophy there were generally only remnants of seminiferous tubules remaining, with absence of most spermatocytes, spermatogonia, and sertoli cells. Interstitial tissues in two of the extremely atrophied testicles contained degenerative changes, including moderate amounts of granular olive-green pigment, generally extracellular, which was non-refractile, non-polarizing, iron negative, and stained slightly orange with the PAS reaction. Eosinophilic bands (collagenous material) were Congo red negative, lightly PAS positive, and stained blue with the trichome stain. These degenerative changes were considered secondary to the atrophy. Histological examination of the epididymis from one affected animal showed no apparent abnormalities.

One of the 12 animals with velvet-covered antlers (a 3-year-old male) had marked infarction of the testes with extensive central coagulative necrosis. Surrounding the coagulative necrosis were a narrow zone of severely degenerative tubules, small amounts of hemorrhage, and small foci of dystrophic calcification. Vascular obstruction of the testes by parasites was not observed microscopically.

Although age differences were not observed between those animals with marked testicular atrophy and those with extreme atrophy, the average age of these two groups combined was approximately 8 (range 3 to 12 years old).

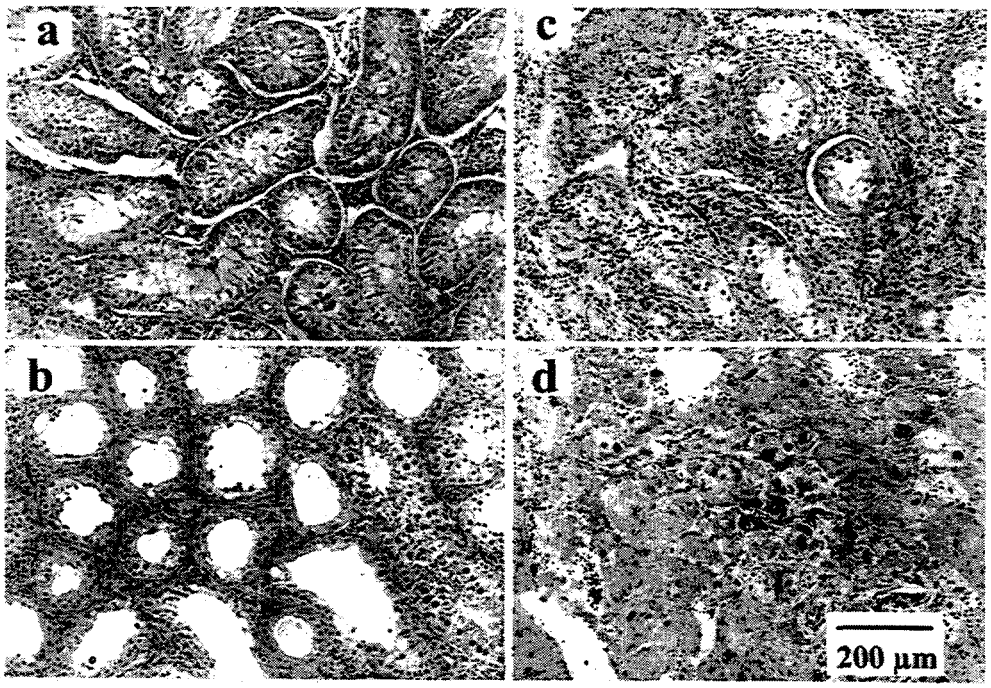


Figure 3.10. Hematoxylin and Eosion-Stained Sections of (a) Normal Testis from a 1-year-old Deer, (b) Testis from a 3-year-old Deer with Slight Atrophy, (c) Testis from a 7-year-old Deer With Marked Atrophy, and (d) Testis from an 8-year-old Deer with Extreme Atrophy

3.6.3 Infectious Diseases

Vascular obstruction of the testes by parasitic infections was not observed histologically in any of the affected bucks. Serological tests for *Brucella* spp. antibodies collected from nine affected animals were negative. Neutralizing antibodies in sera were detected against all of the epizootic hemorragic disease (EHD) viruses (EHDV-1, -2) and bluetongue (BT) viruses (BTV-2, -10, -11, -13, -17), and all but one of 21 deer that tested seropositive for EHDV were seropositive for BTV. Nine (90%) of 10 affected deer had antibodies against BTV-11 and EHDV-2. Six (55%) of 11 unaffected 1-year-old males and six (86%) of seven unaffected males ages 3- to 5-years old also were seropositive for these two viruses (Appendix E).

3.6.4 Sera: Hormones and Constituents

Collection of meaningful physiological data from wild deer can be expensive and difficult to interpret. In addition, a "snap shot" sera sample will not accurately depict seasonal influences, which may have profoundly different results (Seal et al. 1980). However, some general inferences can be made about the animals health and organ functions.

With the exception of blood urea-nitrogen (BUN), serum chemistry results from nine affected and 17 apparently normal animals (Table 3.6) showed no differences between the groups and were essentially

Table 3.6. Mean (± 1 S.E.) Serum Constituents from Affected and Normal Bucks (1993 - 1994)

Serum Parameter	Normal	Affected	Reference[a]
Glucose (mg/dl)	208.7 (9.3)	223.3 (20.2)	60.0 - 320.0
BUN (mg/dl)	29.6 (2.4)	21.1 (2.0)	15.0 - 45.0
Creatinine (mg/dl)	2.2 (0.1)	2.2 (0.1)	0.4 - 2.0
Sodium (mEq/l)	144.1 (1.2)	144.9 (1.8)	132.0 - 156.0
Potassium (mEq/l)	5.3 (0.2)	4.7 (0.4)	3.4 - 5.0
Calcium (mg/dl)	9.7 (0.1)	9.3 (0.3)	8.8 - 10.8
Phosphorus (mg/dl)	6.4 (0.4)	5.1 (0.8)	4.5 - 8.5
T Protein (g/dl)	7.1 (0.3)	6.6 (0.1)	5.0 - 7.8
Albumin (g/dl)	3.7 (0.1)	3.7 (0.1)	2.5 - 4.2
Globulin (g/dl)	3.4 (0.4)	2.8 (0.2)	-
T Bilirubin (mg/dl)	0.3 (0.04)	0.2 (.1)	0.1 - 1.0
Alkaline Phosphatase (iu/l)	84.1 (16.7)	49.1 (3.6)	-
Serum Glutamic Oxaloacetic Transaminase ALT (iu/l)	241.4 (43.9)	145.2 (21.5)	40.0 - 150.0
Chloride (mEq/l)	94.6 (1.1)	95.7 (0.9)	100.0 - 110.0
CO2 (mEq/l)	6.2 (0.8)	9.6 (1.7)	-
Anion Gap	42.7 (1.9)	39.7 (2.9)	-
Cholesterol (mg/dl)	58.9 (3.6)	53.3 (4.8)	30.0 - 100.0
Gamma Glutamyl Transpeptidase (U/L)	216.3 (45.7)	114.9 (17.5)	-
Thyroxine (ug/dl)	10.3 (0.7)	11.3 (0.6)	15.0 - 30.0
Total (n)	17	9	

normal for (*Odocoileus* spp.) (Seal et al. 1981; Wallmo 1981). Blood urea-nitrogen levels were significantly lower ($p \leq 0.10$) in affected animals. Alkaline phosphatase values were slightly elevated in normal animals. Thyroxine levels in Hanford deer were slightly lower as compared to white-tailed deer.

Mean testosterone (T) levels in affected animals were considerably lower than in the normal animals (Table 3.7). Levels of follicle stimulating hormone (FSH) and luteininzing hormone (LH) were more than six and 17 times greater, respectively, in affected animals than in normal animals. All androgen constituents were significantly ($p \leq 0.01$) different between affected and normal animals (Appendix E).

3.6.5 Liver Induction Enzymes

Animals exposed to environmental pollutants respond by inducing liver microsomal phase I and phase II enzymes, which serve to metabolize these xenobiotics. The majority of studies conducted to date have been done with aquatic organisms, especially fish, but a few studies have also documented xenobiotic-dependent induction of phase I enzymes in terrestrial animals (Buhler and Williams 1989; Payne et al. 1987; Stegeman and Lech 1991; Talmage and Walton 1991; Nims and Lubet 1995). The

Table 3.7. Results of Serum Hormone Assays from Affected and Normal Bucks (1993-1994)

	Hormone[a]		
Antler Condition	Testosterone	Leuteninizing Hormone	Follicle Stimulating Hormone
Normal (n = 20)	0.63 ± 0.18[b] (0.09 - 3.36)	0.22 ± 0.03 (0.08 - 0.53)	23.32 ± 4.42 (9.46 - 99.61)
Affected (n = 12)	0.22 ± 0.07 (0.03 - 0.87)	3.87 ± 0.56 (1.05 - 6.84)	149.66 ± 32.78 (76.90 - 504.70)

[a] ng/ml serum.

[b] mean ± 1 standard error (range).

most important phase I enzyme is the cytochrome P450 (CYP) superfamily of monooxygenases (Okey 1990). With respect to pollutants most often encountered in the environment (PAHs, polyhalogenated biphenyls, dioxins, and dibenzofurans), the CYP most often induced and the most reliable biomarker of exposure is CYP1A1. In addition, many environmental chemicals induce the phase II enzymes glutathione S-transferases, which serve to conjugate electrophilic xenobiotics (Rushmore and Pickett 1993; Mannervik and Danielson 1988). Results of total microsomal CYP levels for the deer samples analyzed are shown in Table 3.8.

Little or no information on basal levels of CYP in deer populations is available. The specific contents shown in Table 3.8 are in the range observed for most mammals. No obvious induction was apparent. A more specific measure of exposure to environmental pollutants is the induction of CYP1A1 mRNA, protein or catalytic activity. Induction was readily apparent as in non-exposed, control animals, CYP1A1 levels are extremely low, usually below the limits of detection. In the present study, CYP1A1 was detected in only a few of the Hanford deer samples, strongly indicating that they have not been exposed to significant levels of PAHs, PCBs, PBBs, dioxins, or dibenzofurans.

GST activity can be induced by environmental pollutants of a different class than those which typically induce CYP1A1. GST activities are markedly enhanced after exposure to a number of insecticides such as DDT, chlordane, kepone, and mirex, as well as various herbicides and plasticizers. The GST activity levels for the deer liver cytosols are also shown in Table 3.8.

As with the microsomal CYP specific content data, the lack of information on basal GST levels in deer make interpretation somewhat difficult, but no obvious difference between samples was apparent and in our experience, it is unlikely that any of the deer were exhibiting xenobiotic-dependent induction of GST activity.

Table 3.8. Results of Total Microsomal CYP 1A1/1A2, CYP, and GST Levels From Liver Samples of Affected and Normal Deer

Animal ID	Age	Cyp1A1/1A2[a]	CYP[b]	GST[c]
Atrophy				
148.930	8	N.D.	0.532	206.3
148.900	12	N.D.	0.373	241.5
148.090	14	N.D.	0.385	202.9
149.920	3	4.2	0.241[d]	na
149.960	4	2.7	na	na
Buck X	3	6.9	0.286[d]	na
Mean (+/- 1 S.E.)	7.3 (1.9)	4.6 (1.2)	0.43 (0.05)	216.9 (12.3)
Normal				
148.710	6	N.D.	0.526	255.7
north#1	6	N.D.	0.433	270.3
north#2	4	N.D.	0.443	299.7
148.370	9	N.D.	0.472	243.9
148.130	3	2.7	0.489	na
Mean (+/- 1 S.E.)	5.6 (1.0)	2.7 (na)	0.47 (0.02)	267.4 (12.0)
Oregon				
#1	1	7.9	0.622	na
#2	2	9.6	0.616	na
#3	2	8.3	0.525	na
#4	1	7.8	0.444	na
Mean (+/- 1 S.E.)	1.5 (0.3)	8.4 (0.4)	0.55 (0.04)	

[a] Units expressed as arbitrary densitometry units.
[b] Units nmol/mg protein.
[c] Specific activity (nmol product formed per min, mg protein.
[d] Results indicated the sample was slightly denatured and was not included in analyses.

3.7 Contaminant Levels in Deer

A few normal males who were captured in 1993 and 1994 survived and remained normal (typical, calcified antlers) until they were relatively old, providing an opportunity to sample and analyze contaminant levels in some normal animals with similar ages as the affected males, as described in the following subsections.

3.7.1 Metals

Selected heavy metals have been reported to induce various abnormalities in mammalian testes (Corrier et al. 1985; Ernst 1990; Colburn et al. 1993). Corrier et al. (1985) provided evidence that dietary cobalt-induced degenerative necrotic lesions in the seminiferous tubules of rats. Ernst (1990) studied the effect of tri- and hexavalent chromium injected in the rat and found the hexavalent form

induced testicular atrophy and reduction in epididymal sperm number. These effects were also complimented with significantly reduced body weights. Mason et al. (1964) describe effects of cadmium-induced injury of the rat testis and also document the presence of vascular lesions, interstital edema, and hemorrhage. Colburn et al. (1993) also identified literature documenting the potential for cadmium, lead, or mercury to act as estrogenic-mimicing chemicals. All these papers, however, are experimental in nature and only discuss the effect of certain dose rates over time. In an epdemiologic investigation such as this one, tissue residue levels of the contaminants are the only practical measurable items. In this light, we collected tissue samples from several normal males of similar ages and compared the contaminant levels in those animals to the levels observed in some of the affected males. In addition, tissue samples were collected from deer residing near Boardman, Oregon, and analyzed for the same contaminants to serve as a reference.

Metals found in liver samples collected from six normal (mean age 5 years) and six affected (mean age 7.3 years) animals were similar ($p \geq 0.1$), and with the exceptions of chromium, nickel, and cadmium, were less than or equal to metal levels found in four deer liver samples collected from near Boardman, Oregon (Table 3.9, Appendix F). Boardman deer samples contained elevated ($p \leq 0.0001$) levels of copper as compared to all Hanford deer samples.

Table 3.9. Mean (± 1 S.E.) Levels of Metals Found in Liver Samples Collected from Affected and Normal Bucks (1993 - 1996)

Contaminant	Normal	Affected	Reference[a]
Silver (0.22)[b]	ND	ND	ND
Arsenic (0.83)	ND	ND	ND
Berillium (0.15)	ND	ND	ND
Tin (0.19)	ND	ND	ND
Selenium (1.31)	ND	ND	ND
Thallium (0.04)	ND	ND	ND
Lead (0.08)	ND	ND	0.08 (0.06)
Mercury (0.001)	0.01 (0.006)	0.01 (0.007)	0.01 (0.001)
Chromium (0.07)	0.25 (0.7)	0.36 (0.4)	ND
Nickle (0.056)	0.4 (0.2)	0.4 (.1)	ND
Cadmium (0.08)	0.71 (0.1)	0.76 (0.2)	0.33 (0.04)
Copper (1.20)	56.0 (16.1)	62.1 (16.2)	143.5 (8.9)
Zinc (1.4)	119.5 (10.7)	148.1 (10.3)	132.9 (10.4)
Iron (3.0)	241.4 (43.9)	145.2 (21.5)	NA
Sample Size	6	6	4

[a]Samples collected from near Boardman, Oregon.

[b]Analytical detection limits, units ug/g dry wt.

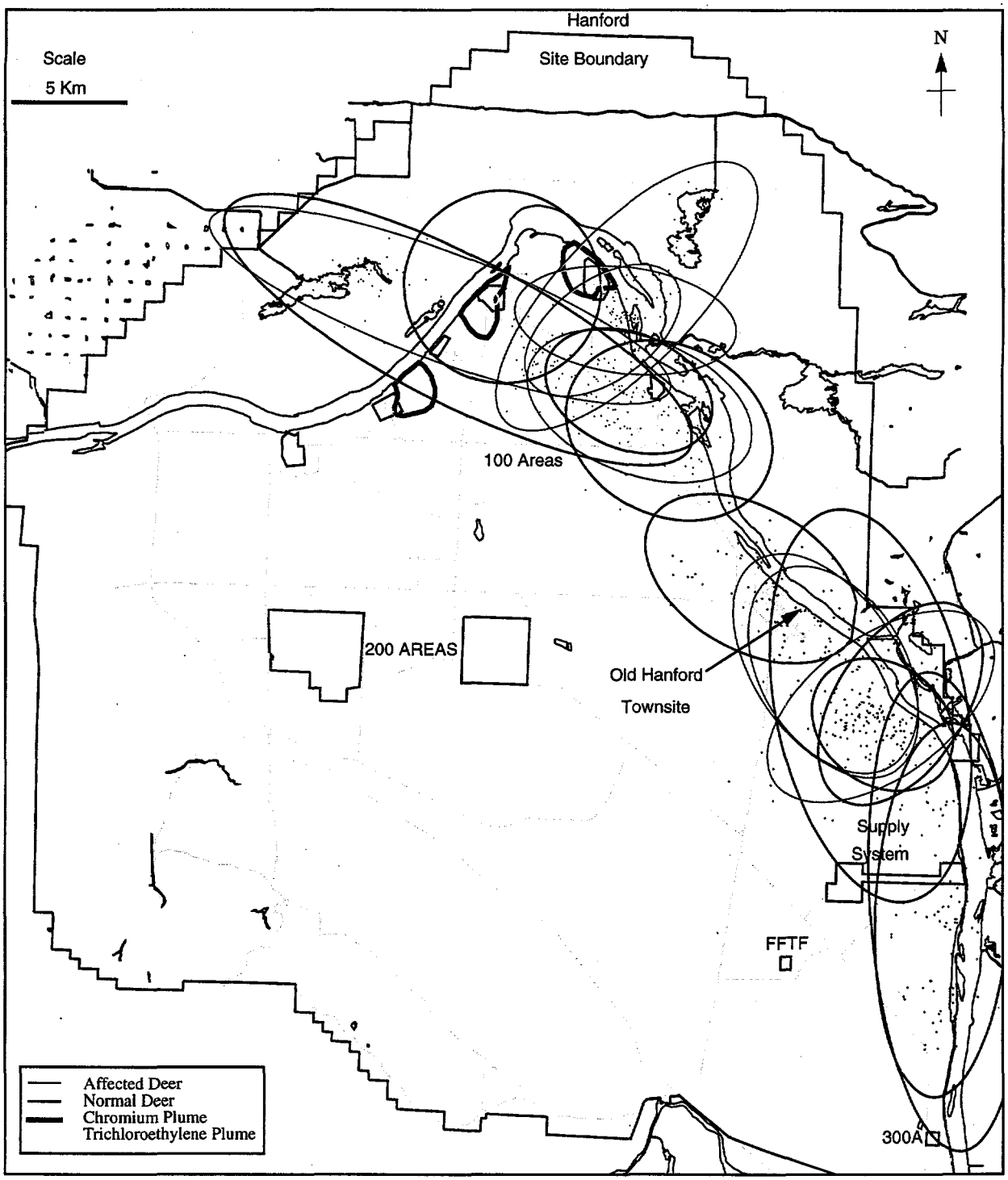


Figure 3.11. Home Ranges (circled areas) and Locations (black dots) of Affected Deer in Relation to Chromium and TCE Plumes on the Hanford Site

Because deer diets suggest substantial consumption of woody species (deep-rooted plants), we also examined the spatial relationships of affected and normal animals with known contaminant plumes on the Hanford Site by counting the number (proportion) of affected animals whose home range was found to contain areas with shallow (approximately 10 m below ground surface) groundwater chromium and tri-chloroethylene (TCE) plumes (Figure 3.11) (Dresel et al. 1996). Results for chromium plume analyses demonstrated that only four of eight affected animal's home range overlapped these areas. Although some animal's home ranges overlapped to some degree with the chromium and TCE plumes, four of the affected animal's home ranges were never found within either the TCE or chromium contamination plumes. Furthermore, the animals whose home ranges overlapped the chromium plumes were never actually found within the contaminant plume area.

3.7.2 Organics

Before establishment of the Hanford Site, agricultural crop production activities were common on lands near the Columbia River. In those days, many compounds used to control insects, invasive plants, and fungi on crop plants have been shown be quite persistent in the environment and can cause effects in animal reproduction (Colburn et al. 1993; Guillette 1994).

In the past 10 years, Hanford has commonly used over 50 compounds to control vegetation in and around operational units and roads, primarily to prevent wildlife intrusion into radiological contaminated areas. In addition, as shown in Figure 3.11, home ranges (and many of the animal's actual locations) of all affected animals extend across the river to areas where agricultural settling ponds exist from active agricultural practices on adjacent private lands.

Polychlorinated biphenyls are a family of chlorinated aromatic compounds that vary widely in physical, chemical, and biological properties. These compounds were patented in 1881 by Schmidt and Schultz; however, PCBs were not commercially used until 1930 in the United States (Connell and Miller 1984). Prior to the 1970s, PCBs were used for a many things: reduction of dusty roads, hydraulic fluid, plasticizers, adhesives, heat transfer systems, lubricants, inks, and a variety of others. Today, federal regulations limit the use of these compounds to electrical capacitors and transformers, vacuum pumps, and gas transmission turbines. However, the persistence of the compounds in the environment surpass the restrictions imposed during the 1970s. PCBs are now ubiquitous in the environment, and monitoring the levels and biological effects are ongoing. Human and terrestrial wildlife exposures to PCBs have resulted largely from consumption of foodstuffs (McKinney and Waller 1994). Much literature is now available that documents the estrogenic effects of these polycyclic aromatic compounds (see Colburn et al. 1993, 1994; McLachlan 1993).

Until recently, relatively little attention was paid to non-radiological contamination on the Hanford Site (Blanton et al. 1995; Dirkes and Hanf 1996). For this reason, samples of brain tissue were collected from nine deer in 1994 and 1995 and assayed for a variety of herbicides and pesticides, including the more persistent and potentially toxic heptachlor, aldrin, 2, 4'-DDE, dieldrin, and endosulfan. In addition, 24 PCB congeners commonly found in PCB-contaminated soils were also analyzed (see Appendix F).

Pesticides 4,4'-DDE, and endosulfan sulfate were detected (0.71 and 0.62 ng/g wet wt., respectively) in one animal exhibiting degenerative testes and velvet-covered antlers. Levels of all other constituents were less than the analytical detection limits. Results from the remaining animals (three affected animals as determined by microscopic examination of the testes, one normal male from the Hanford Site, and four normal males from Boardman, Oregon) were all found to be less than the analytical detection limits.

3.7.3 Radiological Analysis

Extensive radiological surveillance has been performed on the Hanford Site by PNNL and Site operations contractors (Hanf and Dirkes 1995). These surveillance activities provide data to help evaluate the potential for radioactive materials to cause testicular atrophy in Hanford Site deer. Plutonium production activities ceased in 1988 with the shut down of the 100-N reactor, and since that time, all short-lived radionuclides have decayed, leaving only radionuclides with moderate-to-long half-lives. Those considered likely candidates for potentially accumulating in deer--^{60}Co, ^{90}Sr, ^{137}Cs, radioiodine, and $^{239/240}$Pu--are described below. The persistence of these radionuclides in the Hanford Site environment has been evaluated in several trend reports and special studies (Poston and Cooper 1994; Poston et al. 1995). We have evaluated the presence of these radionuclides in deer samples, soil, and vegetation samples. Gamma spectrometry of muscle samples provides measurement of numerous gamma emitting radionuclides. Europium-154 and ^{155}Eu have moderate half-lives, but have not been measured in deer or other biota samples, and therefore, are not addressed further.

Cobalt-60. Surveillance of ^{60}Co in deer muscle has produced negative results for deer collected from 1983 through 1992. Additionally, ^{60}Co has shown a potential for accumulation only in soil around the 100 areas, and then mean concentrations were at the level of detection in soil and were not detected in routine steppe vegetation samples. A study of riparian vegetation along the 100-N shoreline indicated that some mulberry trees had accumulated low levels of ^{60}Co around 0.03 pCi/g. Most vegetation, however, was below detection (<0.02 pCi/g). There is no specific indication that ^{60}Co would accumulate in reproductive organs as cobalt is uniformly distributed among all tissues with the exception of the liver (Coughtrey and Thorne 1983). Given the generally "less than detection" levels of ^{60}Co in both normal and affected deer muscle samples (Appendix F) and plant samples, there is little indication that ^{60}Co would contribute to elevated doses to deer testes.

Strontium-90. Strontium-90 is a chemical analog to calcium in environmental media, i.e., ^{90}Sr accumulates in calcified hard tissue like antler, bone, or shell. Strontium-90 was measured routinely in deer bone and antler samples (Tiller et al. 1996; Poston and Cooper 1995). Although recent results show favorable agreement in ^{90}Sr concentrations between affected and normal deer (Table 3.10), results for ^{90}Sr in bone dating back to 1990 show considerably higher concentrations in some Hanford deer bones comrade to the 1995 values. These elevated concentrations are believed to indicate that 100 Area deer with access to the 100-N shoreline were feeding on riparian plants that had also accumulated environmentally elevated levels of ^{90}Sr (Antonio et al. 1993). Concentrations of ^{90}Sr in bone and antler appear to have returned to normal levels following the removal of contaminated vegetation form the 100-N shoreline area. Background concentrations of ^{90}Sr in antlers of deer from Silver Lake, Oregon, were higher than those of Hanford Site deer because environmental levels are higher due to historic fallout

Table 3.10. Radionuclide Concentrations in Deer (1990-1996)

			^{90}Sr Antler		^{90}Sr Bone		^{137}Cs Muscle	
			Maximum[a]	Mean[b]	Maximum	Mean	Maximum	Mean
				1990-1994				
Hanford								
	North	Normal	0.93 ± 0.18	0.33 + 0.15 [0 of 11]	58 + 11	7.6 + 9.0 [0 of 13]	0.03 + 0.01	0.01 + 0.004 [13 of 17]
		Affected	0.21 + 0.067	0.16 + 0.049 [0 of 3]		NS[c]		NS
	South	Normal	0.38 + 0.082	0.24 + 0.06 [0 of 8]		NS	0.01 + 0.01	0.00 + 0.019 [13 of 13]
		Affected	0.24 + 0.066		0.22 + 0.058	0.12 + 0.20 [0 of 2]	0.01 + 0.01	0.00 + 0.009 [3 of 3]
Offsite								
	Oregon		4.5 + 0.83	2.1 + 0.63[d] [0 of 10]	0.13 + 0.041	0.11 + 0.015[e] [0 of 4]	0.03 + 0.03	0.01 + 0.01[e] [3 of 4]
	Steven's Co.[f] Washington			NS	2.1 + 0.41	1.1 + 1.0 [0 of 3]	0.5 + 0.06	0.31 + 0.26 [0 of 3]
				1995-1996				
Hanford								
	North	Normal		NS	1.6 + 0.30	0.63 + 0.41 [0 of 6]	0.02 + 0.02	0.01 + 0.006 [6 of 7]
		Affected		NS		NS		NS
	South	Normal		NS	0.42 + 0.10	0.32 + 0.15 [0 of 3]	0.01 + 0.02	0.01 + 0.001 [3 of 3]
		Affected		NS	0.49 + 0.11	0.33 + 0.22 [1 of 4]	0.01 + 0.01	0.00 + 0.009 [4 of 4]

[a] Concentration ± 2 sigma propagated analytical error.
[b] Mean ± 2 SEM, [number < detection of total sample number].
[c] Tone sampled.
[d] Silver Lake, Oregon.
[e] Boardman, Oregon.
[f] Whitetail deer (*O. virginianus*).

levels. The summer range of Silver Lake deer is on a mountain, which has a higher annual precipitation (Tiller et al. 1995). Because of the rapid attenuation of beta particles, most of the dose from ^{90}Sr is localized in the bone matrix with little dose reaching the testes in deer.

Coughtrey and Thorne (1983) suggest that 0.01 of accumulated strontium ingested by mammals was distributed in soft, non-calcified tissue. Testes and seminal vesicles had the lowest distribution factors of soft tissue. As a beta emitter, radiation effects are localized at the point of accumulation (primarily bone). Strontium-90 is rarely measured in deer muscle; consequently, the accumulating in testes is equally unlikely.

Cesium-137. Levels of ^{137}Cs are sporadically measured in deer muscle. Levels of ^{139}Cs in background deer samples collected from Stevens County were higher than levels in Hanford deer because of historic patterns of fallout weapons testing (Poston and Cooper 1995). ^{137}Cs accumulates in testes at level commeasurable with the liver. There is an indication that muscle tissue ^{137}Cs accumulates in muscle tissue at levels slightly higher that other soft tissue (Coughtrey and Thorne 1983). The levels of ^{137}Cs observed in

deer muscle do not suggest that ^{137}Cs is source of significant radiation exposure to deer testes. Moreover, ^{137}Cs in vegetation and soil in the home ranges of these deer are at background or less than detection (<0.02 pCi/g) concentrations.

Iodine. Both ^{129}I and ^{131}I were produced as fission products at Hanford. Because of ^{131}I's short 8-day half-life, it is not considered as a source of radiation exposure for deer and hasn't been produced at Hanford since N reactor shut down. Releases of ^{129}I to the atmosphere in the mid-1980s increased with the start-up of PUREX, and decreased when PUREX was permanently shutdown in 1990. Iodine-129, however, is a persistent and mobile radionuclide with an exceedingly long half-life. Rickard and Price (1984) reviewed the environmental significance of iodine at Hanford and reported concentrations of ^{129}I in Hanford deer thyroids in 1983 ranging from 19 to 51 pCi/g. The estimated dose of a 1.8-g thyroid with an effective radius of 1.4 cm is 0.16 urad/d or 57 rad/year based on 51 PCi ^{129}I /g (Baker and Soldat 1992). This incremental increase in dose can be compared to the normal background whole animal dose rate of about 300 mrad/year and is considered insignificant. Also, because sera levels of thyroxine (Table 3.10) and thyroid weights (Appendix F) suggest no difference between affected and normal animals, and are essentially normal for *Odocoileus*, alteration of the thyroid caused by accumulation of radio-iodine deposition is doubtful.

Plutonium. Plutonium has been routinely monitored in deer and rabbit livers during routine surveillance collections and was not measured from 1983 through 1992 in any liver samples (Poston et al. 1994). Liver is the organ of choice as it would accumulate the highest levels of ingested plutonium. The apparent lack of any positive measurement of plutonium in jackrabbit or deer liver (Poston and Cooper 1995) suggests that environmental exposure to plutonium at Hanford is minimal, and consequently, the likelihood of an elevated dose from plutonium affecting testicular development in deer is very remote.

Considering levels of radionuclides found in these animals, radiation exposure is not a plausible explanation for testicular atrophy in deer at Hanford. Radiation exposure is also not likely the cause of testicular atrophy because of the severity of the lesions and absence of effects histologically observed in other tissues (Wilkinson 1969; Fajardo 1982; Jones and Hunt 1983).

4.0 Conclusions

This section presents general conclusions from our examination of testicular atrophy patterns in Hanford Site mule deer.

4.1 General Conclusions

Although low fawn ratios were observed in this area, body weights, sera chemistry, and clinical observations demonstrated no signs typical of deer on an inadequate diet. Furthermore, deer densities on Hanford are relatively low and are reflective of scare semi-arid community resources like succulent forage and water.

In nearly all deer populations, the mortality rate of fawns is greater than that of adults. Even among stable or increasing populations, poor fawn survival is common (Wallmo 1981). Both neonatal (in utero) and/or postnatal (after birth) mortalities can cause low fawn production rates. Eberhardt et al. (1979) believed the Hanford Site generally provided poor nutrient quality for deer, which can cause significant neonatal losses (Wallmo 1981). However, body weights and sera chemistry results suggested healthy animals during the spring period. Steigers (1978) studied postnatal mortality of mule deer fawns on the Hanford Site and found that coyotes (*Canis latrans*) were a major predator. Coyote control programs ceased on the Hanford Site in 1970 (Eberhardt et al. 1979).

Population estimates indicated, on average, 330 individual animals reside within the river region of the Hanford Site. Using the average adult mortality rate (11% per year for males and 5% per year for adult females) and the average ratios of males:females:fawns (47:100:26) found in this region, (see Table 3.1), we calculated that a total of 10 adult male and three adult female deer are harvested in any one year.

The prevalence of testicular atrophy in Hanford Site adult male deer (23%) was greater than any reported to date. This value, however, is difficult to compare with other mule deer populations because of differences in demography. Although some deer hunting occurs along the boundaries of the Hanford Site, the age-class distribution of our study population is unlike most deer populations where 5-year-old males are a rarity (Zeigler 1978; Mendin and Anderson 1979; Story and Kitchings, 1994; Raedeke 1995). Furthermore, <1% of affected animals observed on the ALE Reserve may be correlated with fewer old-aged animals found there because of the relatively high hunting pressure on adjacent lands compared with Columbia River portions of the Hanford Site. Age-class information is crucial for further understanding and relating this phenomenon to other wild mule deer populations.

The epidemiological data and microscopic lesions indicated that testicular atrophy was due to a disease or diseases rather than failure of the testes to develop. This fact alone negates the likelihood that antler morphometry and infertility seen in some male deer on Hanford are linked to an altered genetic expression (Ralls and Ballou 1983). Smith (1986) described a genetic relationship to testicular atrophy in goats. Sperm granulomas are frequently associated with polled homozygus goats; however, no granulomas were found in these deer and histology of epididymis showed no abnormalities. The 1-year-old males essentially were normal, intermediate lesions were present in a 3-year-old buck, and severely

degenerative/atrophic testes occurred in the 5- to 12-year-old males. If we assumed that the bucks we examined were representative of deer in their respective age groups, progressive testicular degeneration has occurred in this deer population. Slightly reduced average testis weights of the Hanford deer exhibiting essentially normal antlers and testes is undoubtedly related to differences in the sampling season as the reference samples were typically collected in fall during deer harvest season whereas we collected samples of testes at the time testicular activities are minimal for mule deer (Brown 1983).

It does not appear the abnormality expressed in some of the older male deer is related to a shorter life span. In fact, it could be postulated that since the affected males are not reproductively active, their life-span is lengthened because they do not expend the copious amount of energy for breeding (rut, battles, etc.) as male deer typically do (Wallmo 1981).

Because reproductive effects often occur in the younger, more sensitive animals, one theory postulated includes the occurrence of some unknown event that took place when the affected animals were young that resulted in the condition being incidentally expressed at older ages. The theory applied to this deer herd is not complete as one radio-tagged deer (148.140) changed from normal to affected in 1995.

Wildfire and subsequent potentiation of toxins/estrogens in plants appears not to be the primary cause of testicular atrophy seen in some of the Hanford Site deer. This point is substantiated because wildfires mapped using GIS that illustrate areas burned in the last 10 years on Hanford do not include home range areas of some affected deer. Furthermore, mean serum hormone levels of FSH and LH found in the affected animals were more than six and 17 times, respectively, higher than those found in normal animals. The levels demonstrate proper feedback responses of the gonadopituitary axis to extremely low levels of T found in affected animals. This indicates the causative agent(s) are acting directly on testicular tissues rather than an indirectly altered hormone mechanism, as might occur with plant estrogens.

Although Blanchard et al. (1991a) discuss senile testicular atrophy as a condition that varies by species, this condition has not been reported in captive deer herds containing 5- to 10-year-old males. Steinhoff (1957) recorded antler status for a captive mule deer buck for all 18 years of life and found typical calcification of the antlers up to its seventeenth year of life. At that point, antlers developed as "small and decadent, shaped like a pin cushion with many small velvet-covered protuberances."

Radiation exposure is not likely the cause of testicular atrophy because of the severity of the lesions and absence of effects in other tissues (Wilkinson 1969; Fajardo 1982; Jones and Hunt 1983). Radiological constituents were not different ($p > 0.10$) between affected and normal animals on the Hanford Site and do not indicate excessive levels (Dirkes and Hanf 1996). Also, because sera levels of thyroxine and fresh weights of thryroids suggested no difference between affected and normal animals, and are essentially normal for *Odocoileus*, alteration of the thyroid caused by radio-iodine deposition is doubtful. In addition, radiation exposure measurements on the Hanford Site indicated that deer would not be exposed to levels producing even slight changes in testicular functions (IAEA 1992; Woodruff and Hanf 1992). Even under significantly conservative and unrealistic exposure scenarios, the estimated dose rates to deer in the N-springs area were estimated at 0.08 rad/d (Poston and Soldat 1992). For comparison, IAEA (1992) has indicated that an environmental dose rate of 1.0 rad/d was a threshold level for the appearance of population effects. It should also be noted that at lower doses rates, 0.2 rad/d

reduced sperm numbers, motility and viability in dogs. The no effect level was <0.12 rad/d. Using these points for reference, there appears to be no relationship between environmental levels of radionuclides at Hanford and testicular atrophy in deer.

Testicular atrophy caused by elevated levels of selected heavy metals also does not appear likely in the Hanford deer. Chromium levels were similar among normal and affected animals, and literature relating testicular damage to acute chromium poisoning also documents a loss of body weight and renal tubular necrosis (Driver 1994; Eisler 1986). Histological examination of the kidneys from affected deer showed no signs of kidney dysfunctions and both affected and normal buck whole body weights were not different and were typical of Rocky Mountain mule deer (Robinette et al. 1977). Furthermore, use of the TCE- (although not a metal) and chromium-contaminated areas on the Site was not a common denominator for all affected deer. Cadmium toxicity of the testes causes hemorrhage-related lesions and is unlike those observed microscopically in testes collected from affected deer on the Site. Cobalt also is known to have a direct degenerative effect on the mammalian testes; however, this element is found only as a radio-isotope contaminant on the Hanford Site (Napier et al. 1995) and, essentially, was not detected in any affected or normal deer. Long-term studies of the effects from low-levels of chromium warrant further research.

Colburn et al. 1993 lists 45 environmental contaminants or classes of agents that have been reported to cause changes in reproductive and/or hormone systems. McLachlan (1993) and the book "Our Stolen Future" by Colburn et al. (1992) further explores the causes and implications of this recent concern. Generally, these hormone-mimicing contaminants include herbicides, fungicides, insecticides, nematocides, and PCBs.

The basic premise of "endocrine-mimicry" lies in the fact that living organisms cannot distinguish their own hormones from many human-made compounds. As a result, the contaminants may either permanently or temporarily block hormone receptor sites in the body that play a role in reproduction. In the testes, a number of hormonally regulated actions occur. As a simple example, LH and FSH are produced by the anterior pituitary and are secreted into the bloodstream when abnormally low levels of T are detected by the hypothalamus (Vander et al. 1970). Luteinizing hormone and FSH stimulate the growth of the interstitial cells and the secretion of testosterone from these cells located between the seminiferous tubules in the testes. If an endocrine-mimicing were present in sufficient amounts, its presence in the body could (falsely) indicate sufficient amounts of T are present to maintain testicular functions. As a consequence, LH and FSH secretion rates from the pituitary would be set for the exogenous compound levels and not T, causing these T-producing cells to atrophy.

Mean serum hormone levels of FSH and LH found in the affected animals were more than six and 17 times, respectively, higher than those found in normal animals. The levels demonstrate proper feedback responses of the gonadopituitary axis to extremely low levels of T found in affected animals. The elevated pituitary weights from the Hanford deer seems plausible because we know the affected animals demonstrate high releases of LH and FSH into the bloodstream. These results suggest testicular tissues have been directly affected by the causative agent rather than by altered hormonal pathways, as would occur from natural or human-made estrogen-mimicing compounds. Levels of PCBs and a variety of pesticides were not detected in three of four affected animals. Sera samples from affected deer were taken only during the spring and might not indicate levels during other seasons.

The lack of any obvious significant differences in total liver microsomal CYP specific content or in liver cytosolic GST activity between individuals is indirect evidence that these animals have not been exposed to significant levels of organic xenobiotics. The lack of any detectable CYP1A1 expression in the liver microsomes from these animals provides more conclusive evidence for lack of exposure to environmental pollutants, at least to those known to induce CYP1A1 (PAHs, PCBs, TCDD, TCDF). It is doubtful that the failure to detect CYP1A1 protein in any of the deer liver samples was due to a lack of immuno-cross-reactivity between the antibody to rat CYP1A1 and the deer liver ortholog. The identity of CYP1A1 across species is very high (80-90%) and we have successfully utilized such antibodies on western blots to detect CYP1A1 in species as widely divergent as humans and trout.

Although the causes of testicular degeneration in domestic animals are numerous (McEntee 1990), the degree of severity in the older bucks on Hanford generally exceeded that observed with nutritional disorders and, for the most part, poisons. Robinette et al. (1977) observed that the lack of velvet shedding among yearling mule deer in Utah was associated with their physical condition. Seven percent of the hunter-killed yearlings in 1949, which followed the most severe winter of their study, retained most of their velvet. However, atrophic testes were not associated with the deer exhibiting velvet-covered antlers. A diet adequate for normal growth and maintenance is usually also satisfactory for reproduction. The effect of trace mineral and vitamin deficiency is largely postulation. Vitamin A and E deficiencies may be expected to cause testis atrophy (Smith 1986). Since deficiencies have also resulted in atrophy of testes but is also complimented with weakness and dry hair coat. With only one exception, the affected deer on Hanford showed no signs of poor pelage. Considering the dramatic changes in climate and forage for deer throughout the year, further studies are needed to completely rule out nutritional stresses as a cause of testicular atrophy in this herd. No differences in pelage or general body condition were observed between affected and normal animals captured in this study and the results of serum chemistry analyses were essentially normal. The "reference" values and for comparison in this study include samples taken at different times of the year and are from different deer populations (Seal et al. 1980).

The difference between affected and normal animal serum alkaline phosphatase levels likely is related to antlerogenesis. Increased levels of alkaline phosphatase in serum presumably play a role in the antler shedding and growing processes (Molello et al. 1963). The lower alkaline phosphatase values found in affected animals are probably because they do not shed their antlers. Comparing alkaline phosphatase values between affected and normal animals also contains significant age-related differences that have been inversely correlated with serum levels of alkaline phosphatase (Seal et al. 1978).

There was no evidence of the brucellosis in the bucks, indicating this infectious disease process was not responsible for the atrophic testicles. Parasites were not microscopically observed within the testicular vessels, and therefore, were not likely responsible for the atypical condition found in some Hanford Site bucks.

4.2 Areas of Continued Interest

As with any epidemiologic investigation, all aspects of herd health should be considered. Blanchard et al. (1991a) reports one of the most common causes of testicular degeneration in large domestic animals (bulls and rams) is thermal stress. However, scrotal temperatures (2° to 8°C) above normal temperatures show decreases in semen but typically return to normal (Blanchard et al. 1991b). Byers and

Glover (1984) demonstrated high levels of the FSH and LH and low testosterone concentrations in a ram scrotal insulation study. Scrotal insulation resulted in a decrease in the number of germ cells in the seminiferous tubules within 28 days after elevating the scrotal temperature. Ambient summer temperatures on the Hanford Site can exceed 43°C (110°F) and represent another rather unique condition of the resident mule deer (Dirkes and Hanf 1995). Sargent et al. (1994) studied thermoregulation by mule deer on the ALE Reserve and found internal body temperatures to be similar to those reported for *Odocoileus*, however this condition (atrophy) is rarely expressed in the ALE deer herd.

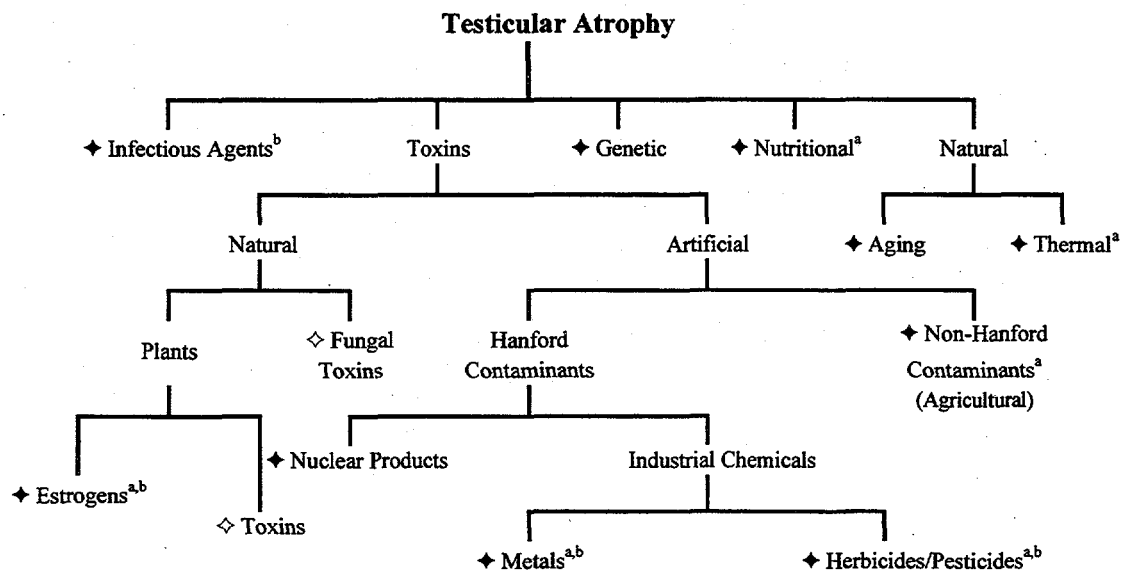
There appeared to be a positive correlation between age and seroprevalance to BTV or EHDV. Most study animals exhibiting testicular atrophy were relatively old males, and the majority of them were seropositive. Notably, one affected animal with extreme degeneration of the testes did not have antibodies for BTV or EHDV. Although signs typical of hemorrhagic disease have never been observed in the Hanford Site deer and are not likely the primary causative agents, the viruses still might play a role in the etiology of testicular atrophy in *Odocoileus*. Short-term experimental studies of EHDV and BTV in deer have demonstrated striking changes in vascular endothelium, and virus replication may initiate intravascular thrombosis (Karstad and Trainer 1967; Tsai and Karstad 1973). Because testicular arteries are relatively long and thin, EHDV or BTV conceivably could result in reduced or completely blocked blood flow to the testes, producing degenerative and/or infarcted tissues. The answer awaits long-term experimental studies on captive deer.

Mycotoxins are described as a large group of compounds produced by a variety of fungal species world-wide. Molds will incubate and colonize on vegetative matter, which can then be inadvertently ingested by humans or animals and can produce toxic effects (Sharma and Salunkhe 1991), including testicular atrophy. Fenske and Fink-Gremmels (1990) demonstrated in-vivo a direct effect (reduction of testosterone production) in rat testis when given the mycotoxin zearalenone, presumably by inhibiting the early steps of spermatogenesis. Colburn et al. (1993) also identified zearalenone as a potent estrogen analog with subsequent reproductive impairment in mammalian cells. Although no data concerning fungal growth on Hanford's plants exist, we frequently see "smut" or fungal growth on the shoots of several grasses commonly consumed by deer on the Hanford Site.

Experimental toxicity studies of the herbicide sulfometuron methyl, tradename "Oust" has shown that this chemical can directly produce testicular degeneration/atrophy in both rats and dogs (EPA 1981; EPA 1983; Seyler et al. 1992). Oust has been used extensively on the Hanford Site and within areas occupied by affected deer through 1996. Persistence (half-life in soils) of the chemical ranges from 8 to 24 weeks, providing an opportunity for deer to consume contaminated plants. Detecting residual levels of this herbicide in selected body compartments is very difficult. It is also possible to have missed the seasons when animals ingested a substantial dose of the causative xenobiotic, hence, missing the elevated levels of liver induction/conjugation enzymes.

It should be noted that state and federal standards regulate the storage and use of herbicides at Hanford. In 1995, Hanford was in compliance with all standards administered by the Washington State Department of Agriculture to regulate the federal Insecticide, Fungicide, and Rodenticide, administered by EPA in Washington State (Dirkes and Hanf 1996). Hanford's biological control program uses integrated pest management criteria to provide an environmentally sensitive approach to pest management (Giddings 1996).

Figure 4.1 shows a logistical flow diagram for potential causative factors responsible for "cactus bucks" on the Hanford Site. This figure illustrates multiple possible reproductive stressors, yet, the combined effect of any two or more these stressors also could exist. Several possible causative agents have been ruled out, but the ultimate cause of testicular atrophy in this deer herd remains unknown.



✦denotes endpoints which have been eliminated as likely primary causes.
✧ denotes endpoints that have not been eliminated as likely primary causes.
[a] warrants field research.
[b] indicates laboratory studies are required.

Figure 4.1. Potential Causative Agents of "Cactus Bucks" on the Hanford Site

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Appendix A

Deer Classifications

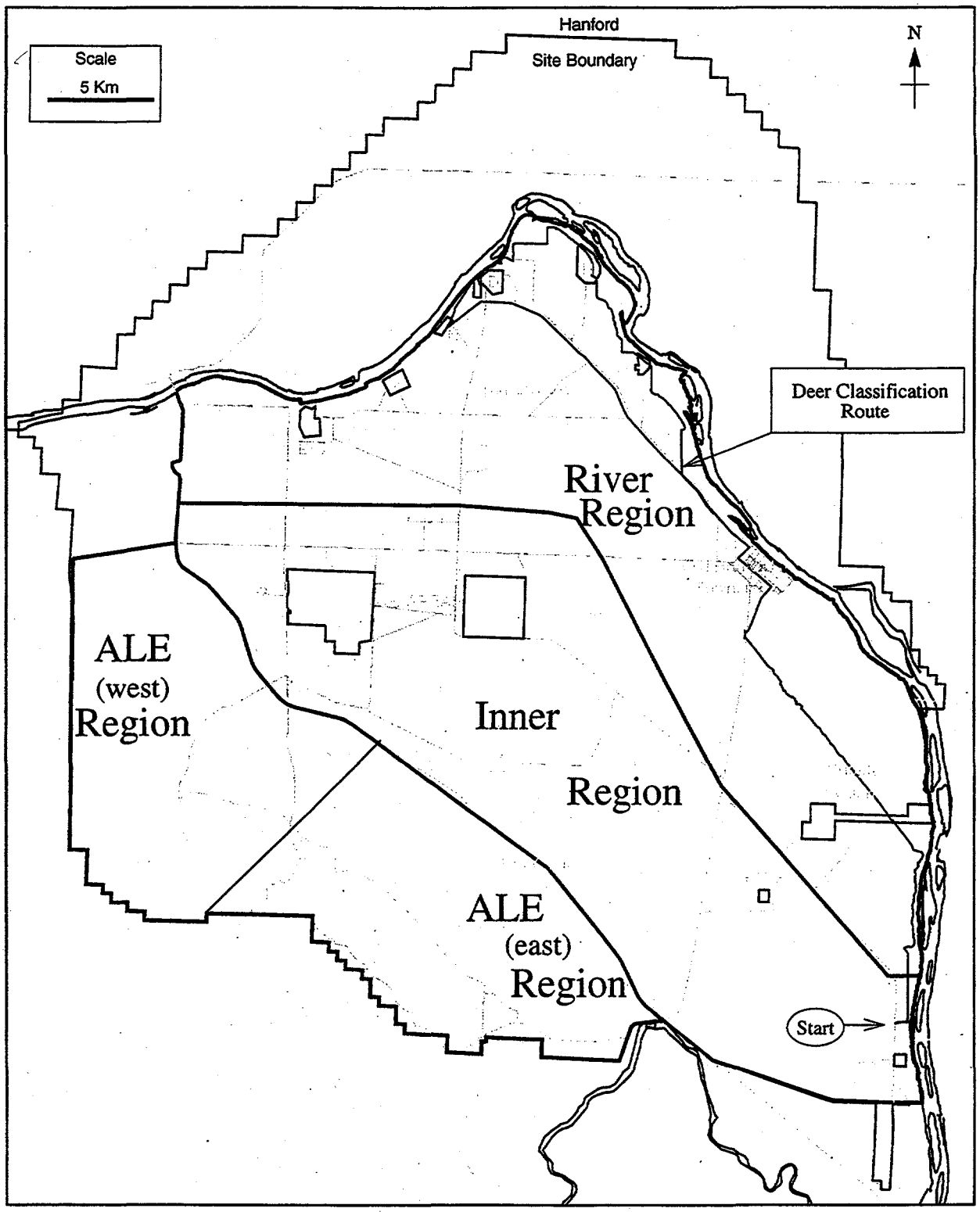

Figure A.1. Hanford Site Stratified by Region Showing Deer Classification Route

Appendix B

Population Size Estimates

Table B.1. Mark-Resight Data Collected During 1994 Surveys on the Deer Classification Route

Survey Date	Number of Does	Marked Does	Deer Id's (Radio-Frequencies)			
9/25/95	14	3	9640	8740	9530	
9/26/95	14	3	9460	8602	8320	
9/27/95	31	4	8640	8740	9530	8380
9/28/95	14	3	8320	9530	9460	
9/29/95	21	2	8320	9530		
10/2/95	24	4	8320	8740	9530	8640
10/3/95	23	4	9588	8640	9530	
10/4/95	15	1	9530			
10/6/95	25	2	9588	9530		
10/9/95	24	3	9588	8640	9530	
10/10/95	22	1	9460			
10/11/95	21	3	9530	8320	9460	
10/12/95	34	3	9588	8320	8740	
10/13/95	30	4	9530	8640	8740	9588
10/16/95	12	0				
10/17/95	27	1	9530			
10/18/95	19	2	9530	9460		
10/19/95	9	1	8502			
10/20/95	30	3	9588	8320	9530	
10/23/95	15	0				

Table B.2. Fecal Pellet Count Results

REGION	PLOT #	UTM		LOCATION	BrMAP Community Category	PELLET GROUPS[a] CHECKED 10/28-11/21/94	PELLET GROUPS CHECKED Apr-95	PELLET GROUPS CHECKED Oct-95	PELLET GROUPS COMBINE 1995
River	1*	306739	5172438	S. 100D	1	15	101	7	108
River	2	311613	5172382	W. White Bluffs	1	42	55	6	61
River	4	313166	5169448	E. 100F	1	33	43	4	47
River	5	318181	5160961	S. HTS	5	79	20	4	24
River	6	314096	5169196	E./NE. 100F/Island	1	36	17	6	23
River	7*	317051	5163011	E. HTS/Island	3	88	19	14	33
River	9	325235	5146532	SE. WPPSS	5	57	nf	6	6
River	10*	324960	5141794	N. 300 Area	1	118	nf	41	41
River	3	314434	5164421	HTS Orchards	2 (avoided)	66	32	3	35
River	8	320668	5146532	Dunes	4 (preferred)	153	37	22	59
Mean						59	43	11	43
Inner	11	307889	5156596	SE. 200E	1	1	0	0	0
Inner	12	310362	5151931	S. landfill	5	0	1	0	1
Inner	14	295930	5165721	W. Gable Bute	1	29	23	7	30
Inner	15*	308703	5158014	E. 200E	1	24	84	1	85
Inner	16	317503	5141957	S. FFTF	5	0	nf	0	0
Inner	13	305117	5148925	N. 240/Gate 112	2 (avoided)	0	0	0	0
Mean						11	27	2	23
ALE	17	291038	5149383	Mouth Snively	3	51	51	0	51
ALE	18	292689	5149837	S. Snively/Hill	5	23	51	2	53
ALE	20*	294294	5149360	S. Snively/Fence	1	53	46	8	54
ALE	21	292884	5153703	Rattlesnake Spr.	1	49	13	14	27
ALE	22	292254	5155452	N. Rattles. Spr.	5	25	5	13	18
ALE	19	293373	5149286	S. Snively/Draw	2 (avoided)	24	8	4	12
ALE	24	296425	5146418	Bobcat Canyon	2 (avoided)	43	31	6	37
Mean						40	33	7	41
ALE (east)	23	287320	5150438	S. Loop Rd.	6	1	0	0	0
ALE (east)	25	305492	5141064	Headquarters	5	4	0	0	0
ALE (east)	26	304699	5147668	W. Gate 112	5	3	0	1	1
Mean						2.7	0.0	0.3	0.3

[a]inital check date (1994) included all old pellet groups.

* indicates deer trail crossings were found within the plot.

(nf) indicates plot was not found.

Appendix C

Habitat Use and Dietary Analyses

Table C.1. Summary of Use Versus Availability Analyses

Vegetation Cover Type		Avoidance[a]		Indiscriminate (avoid)[b]	
		x (n)[e]	%	x (n)	%
1	Post-fire shrube-steppe	0 (11)	0.0%	4 (7)	57.1%
2	CHRYS/Bunchgrasses	2 (4)	50.0%	1 (4)	25.0%
4	ARTR/Bunchgrasses-BRTE	1 (8)	12.5%	1 (8)	12.5%
5	ARTR-GRSP/Bunchgrasses-BRTE	0 (6)	0.0%	3 (6)	50.0%
14	BRTE-POSA	5 (11)	45.5%	4 (11)	36.4%
16	Sand Dunes-PUTR/Bunchgrass	0 (11)	0.0%	2 (11)	18.2%
19	Riparian	1 (8)	12.5%	1 (8)	12.5%
20	Basalt Outcrops	1 (3)	33.3%	2 (3)	66.7%
22	Abandoned Old Fields	0 (12)	0.0%	7 (12)	58.3%
23	Buildings, Parking Lots	2 (7)	28.6%	5 (7)	71.4%
24	Riverine wetlands	3 (15)	20.0%	11 (15)	73.3%
25	White Bluffs	3 (5)	60.0%	2 (5)	40.0%

Vegetation Cover Type		Selection[c]		Indiscriminate (select)[d]	
		x (n)	%	x (n)	%
1	Post-fire shrube-steppe	1 (7)	14.3%	2 (7)	28.6%
2	CHRYS/Bunchgrasses	0 (4)	0.0%	1 (4)	25.0%
4	ARTR/Bunchgrasses-BRTE	0 (8)	0.0%	6 (8)	75.0%
5	ARTR-GRSP/Bunchgrasses-BRTE	0 (6)	0.0%	3 (6)	50.0%
14	BRTE-POSA	0 (11)	0.0%	2 (11)	18.2%
16	Sand Dunes-PUTR/Bunchgrass	0 (11)	0.0%	9 (11)	81.8%
19	Riparian	0 (8)	0.0%	6 (8)	75.0%
20	Basalt Outcrops	0 (3)	0.0%	0 (3)	0.0%
22	Abandoned Old Fields	0 (12)	0.0%	5 (12)	41.7%
23	Buildings, Parking Lots	0 (7)	0.0%	0 (7)	0.0%
24	Riverine wetlands	0 (15)	0.0%	1 (15)	0.0%
25	White Bluffs	0 (5)	0.0%	0 (5)	0.0%

[a] Percent of times the animal was observed within cover type was significantly ($P < 0.05$) smaller than the percent cover type available within 100% M.C.P. home range.

[b] Percent of times the animal was observed within cover type was smaller than the percent cover type available within 100% M.C.P. home range but results were not significant.

[c] Percent of times the animal was observed within cover type was significantly ($P < 0.05$) greater than the percent cover type available within 100% M.C.P. home range.

[d] Percent of times the animal was observed within cover type was greater than the percent cover type available within 100% M.C.P. home range but results were not significant.

[e] number of individuals (# of animals that have at least 3.0% of the cover type availablie within their home ranges).

Table C.2. Dietary Results by Plant Type

SUMMER	Grasses	%	Shrubs	%	Forbs	%
South Region	other	3.0	Artemesia tridentata	< 2.5	Astragalus spp.	2.1
			Atriplex spinosa	< 2.5	Balsamorhiza sp.	< 2.5
			Morus alba	9.0	Descurainia pinnata	< 2.5
			Purshia tridentata	41.8	Oenothera spp.	4.1
			Salix spp.	15.9	Lactuca serriola	< 2.5
			other	2.4	Lupinus spp.	< 2.5
					Melilotus spp.	4.7
					Plantago sp.	< 2.5
					Salsola kali	< 2.5
					Sphaeralcea sp.	< 2.5
					other	15.4
Total %		3.0		69.2		26.3
North Region	other	2.8	Atriplex spinosa	< 2.5	Astragalus spp.	< 2.5
			Morus alba	5.3	Balsamorhiza sp.	1.8
			Elaeagnus angustifolia	34.6	Oenothera spp.	1.5
			Purshia tridentata	12.2	Equisetum spp.	< 2.5
			Salix spp.	17.6	Lactuca serriola	< 2.5
			other	1.9	Lupinus spp.	< 2.5
					Melilotus spp.	3.5
					Penstemon spp.	< 2.5
					Phlox longifolia	< 2.5
					Sphaeralcea sp.	< 2.5
					other	14.3
Total %		2.8		71.7		21.1
FALL						
South Region	Pseudoregnaria spikata	< 2.5	Atriplex spinosa	5.0	Astragalus spp.	2.8
	Aristida spp.	< 2.5	Morus alba	5.2	Balsamorhiza sp.	< 2.5
	Bromus tectorum	4.6	Chrysothamnus spp.	< 2.5	Epilobium sp.	< 2.5
	Muhlenbergia spp.	< 2.5	Purshia tridentata	2.3	Lactuca serriola	3.5
	Oryzopsis hymenoides	< 2.5	Salix spp.	7.1	Opuntia sp.	< 2.5
	Phalaris arundinacea	< 2.5	other	3.4	Phlox longifolia	< 2.5
	Poa sandbergii	31.0			Plantago spp.	< 2.5
	Stipa comata	< 2.5			Rumex spp.	< 2.5
	other	11.6			Salsola kali	2.7
					Sisymbrium sp.	< 2.5
					Solidago spp.	< 2.5
					other	16.8
Total %		47.2		23.0		25.8
North Region	Pseudoregnaria spikata	< 2.5	Atriplex spinosa	2.8	Aster spp.	< 2.5
	Bromus tectorum	3.2	Morus alba	34.6	Astragalus spp.	< 2.5
	Muhlenbergia spp.	< 2.5	Elaeagnus angustifolia	8.0	Balsamorhiza sp.	< 2.5
	Poa sandbergii	3.9	Purshia tridentata	< 2.5	Epilobium sp.	< 2.5
	Stipa comata	< 2.5	Salix spp.	15.0	Oenothera spp.	< 2.5
	other	6.4	other	4.5	Equisetum spp.	2.8
					Lactuca serriola	< 2.5
					Lupinus spp.	2.8
					Melilotus spp.	2.8
					Phlox longifolia	< 2.5
					Plantago spp.	< 2.5
					Salsola kali	< 2.5
					Solidago spp.	< 2.5
					Sphaeralcea sp.	< 2.5
					other	18.3
Total %		13.5		64.9		26.7

Table C.3. Results of Dietary Analysis in Composites

	SUMMER (May, June, July, August)				FALL (Sept., Oct., Nov., Dec.)			
	(n)	% Grasses	% Shrubs	% Forbs	(n)	% Grasses	% Shrubs	% Forbs
South Region								
composite-1	11	3.0	75.9	19.1	9	50.4	19.0	26.6
composite-2	11	2.5	71.4	24.9	9	48.7	23.0	23.8
composite-3	11	3.4	60.3	33.4	9	44.0	25.4	25.1
mean		3.0	69.2	25.8		47.7	22.5	25.2
North Region								
composite-1	11	0.9	74.1	21.6	13	13.9	56.4	27.6
composite-2	11	3.6	74.9	14.8	13	11.3	69.9	16.7
composite-3	11	4.0	66.0	23.3	13	10.1	62.3	27.1
mean		2.8	71.7	19.9		11.8	62.9	23.8

	SPRING (Jan., Feb., Mar., Apr.)			
	(n)	% Grasses	% Shrubs	% Forbs
South Region				
composite-1	7	62.6	5.7	30.6
composite-2	7	59.7	5.5	34.0
composite-3	7	57.5	7.7	34.6
mean		59.9	6.3	33.1
North Region				
composite-1	12	48.7	8.2	42.5
composite-2	12	46.1	5.3	47.9
composite-3	12	69.0	3.3	27.0
mean		54.6	5.6	39.1

Appendix D

Testicular Atrophy

Table D.1. Master Data Sheet for Affected and Normal Animals

AGE CLASS IN YEARS AT CAPT.	ANIMAL ID	CAPTURE METHOD	CAPTURE DATE	CAPTURE AGE	STAGE OF ATROPHY	WHOLE BODY (Kg)	TESTIS WT. (grams)	PITUITARY WT. (grams)	THYROID WT. (grams)
Velvet-Covered									
1-2	\	\	\	\	\		\		
3-4	9960	DRIVE NET	3/21/93	3	INFARCTION	76	4.6		
5-6	8930	NET GUN	2/18/94	5	EXTREME	63	3.5	1.3	2.4
	8020	DRIVE NET	2/04/92	6	ATROPHY (nd)	68	6.5		
	8100	DART GUN	6/1/93	6	EXTREME		4.0		
7-8	9900	DRIVE NET	3/21/93	7	EXTREME		2.5		
	9910	DRIVE NET	3/21/93	7	MARKED-EXT		2.4		
	9932	DRIVE NET	3/21/93	8	EXTREME		1.4		
9-10	8900	NET GUN	2/17/94	9	EXTREME	107	5.0		
	8250	NET GUN	2/19/94	10	MARKED	96	4.7		
11+	8090	NET GUN	2/18/94	11	EXTREME	81	6.5		
	8860	NET GUN	2/18/94	11	EXTREME	68	5.5		
	*8860	DART GUN	12/94	12					
	8980	NET GUN	2/19/94	12	MARKED-EXT	89			
	*8900	Sacrifice	9/17/96	12			5.0	1.1	3.0
	*8090	Sacrifice	9/17/96	14			6.5	1.6	L-0.9 R-1.0
Typical									
1-2	8730	NET GUN	2/17/94	1		68			
	8490	NET GUN	2/19/94	1		50			
	8820	NET GUN	2/17/94	1		89			
	9920	DRIVE NET	3/21/93	3	SLIGHT	68	9.7		
	9920*	Road Kill	3/10/94				20.6a	0.8	L-2.9 R-3.5
	9890	DRIVE NET	3/21/93	1	NORMAL		6.5		
	9970	DRIVE NET	3/21/93	1	NORMAL		7.8		
	#1	SACRIFICE	12/20/94	1					
	#4	SACRIFICE	12/20/94	1					
	8130	NET GUN	2/18/94	2		78			
	8310	NET GUN	2/18/94	2		55			
	8470	NET GUN	2/18/94	2		63			
	8580	NET GUN	2/17/94	2		81			
	8910	NET GUN	2/17/94	2		68			
	8990	NET GUN	2/17/94	2		72			
	9860	DRIVE NET	3/21/93	2	NORMAL		11.9		
	9950	DRIVE NET	3/21/93	2	NORMAL		10.2		
	#2	SACRIFICE	12/20/94	2					
	#3	SACRIFICE	12/20/94	2					
3-4	*8130	HARVEST	10/19/94	3			16.2		
	8430	NET GUN	2/18/94	3		98			
	8970	NET GUN	2/17/94	3		94			
	8710	DRIVE NET	2/5/92	3		76			
	8030	DRIVE NET	2/5/92	4					
	8210	NET GUN	2/19/94	4	SLIGHT	98			
	North #2	Sacrificed	9/18/96	4	NORMAL		35	1.5	1.3
5-6	*8710	DART GUN	12/94	5					
	8370	NET GUN	2/19/94	6		96			
	North #1	Sacrificed	9/18/96	6	NORMAL		40.0	1.4	2.5
7-9	8690	DART GUN	12/16/94	7					
	8190	DART GUN	12/16/94	8					
	*8710	Sacrificed	9/17/96	6			27.0	1.8	L-2.6 R-2.0
	*8370	Sacrificed	9/18/96	9			28.0	0.6	3.5
NO AGE	8120	DRIVE NET	2/4/94						
NO AGE	8140	DRIVE NET	3/15/92						
NO AGE	8810	NET GUN	2/18/94	yrling		41			

a sample wt taken with epidymis attached.
* Indicates Animal has been previously captured.

Table D.2. Antler Observations of Radio-Transmittered Bucks on the Hanford Site

ANIMAL ID	ANTLER DESCRIPTIONS 1992-1993	1993-94	1994-95	1995-96	1996-97
AFFECTED					
9960	Velvet - Cactus			Velvet - Cactus	Velvet - Cactus
8930		Velvet - Cactus		Velvet - Cactus	
8100		Velvet - Cactus			
8900		Velvet - Cactus		Velvet - Cactus	
8020		Velvet - Cactus		Velvet - Cactus	Velvet - Cactus
9900	Velvet - Cactus			Velvet - Cactus	**Velvet - 3 X 4**
9910	Velvet - Cactus			Velvet - Cactus	Velvet - Cactus
8250		Velvet - Atypical			
9932	Velvet - Cactus			Velvet - Cactus	
8090		Velvet - Cactus		Velvet - Cactus	Velvet - Cactus
8860		Velvet - Cactus		Velvet - Cactus	Velvet - Cactus
8980		Velvet - Cactus			
NORMAL					
8730					
8130		2X3	3X4		
8490					
8820		3X3			
9920		2X2			
8310		2X1	2X2		
8470			3X4		
8580		3X3	3X3	4X4	
8910		2X2		3X4	
8990		3X3	2X2		
9860		4X4			
9890					
9950					
9970			3X3		
8430		4X5	5X4	4X4	5X4
8970		4X4			
8030	4X4		5X5		
8210			4X4		
8710	3X4	3X4	2X3	3X4	2X3
8370			2X2	3X4	2X2
8120					
8140		4X4	4X5	**Velvet - Two Pt.**	
8190			2X2		
8690			4X3		
8810					
#1			2X2		
#2			3X2		
#3			4X5		
#4			3X2		

Appendix E

Microscopic and Physiologic Sampling and Analysis

Table E.1. Individual Results of Androgen Sera Analyses

ANIMAL ID	TAG #	CAPTURE METHOD	CAPTURE DATE	LOCATION	LH NG/ML	FSH NG/ML	TEST. NG/ML
AFFECTED							
9960	214	DRIVE NET	3/21/93	HTS	4.3	140.59	0.17
8930	293	NET GUN	2/18/94	100F	3.19	108.92	0.29
8100		DRIVE NET	2/4/92	100D			
*8100		DART GUN	6/93				
8900	252	NET GUN	2/17/94	DUNES	5.96	128.68	0.29
8020	270	DRIVE NET	2/4/92	100D	3.05	112.23	0.11
9900	205	DRIVE NET	3/21/93				
9910	220	DRIVE NET	3/21/93	HTS			
8250	266	NET GUN	2/19/94	100N-K	6.63	122.53	0.87
9932	SILVER	DRIVE NET	3/21/93	HTS	0.12	13.2	0.19
8090	259	NET GUN	2/18/94	100F	4.54	108.06	0.03
8860	276	NET GUN	2/18/94	100F	2.18	128.52	0.32
*8860		DART GUN	12/94	W. BLUFFS			
8980	269	NET GUN	2/19/94	100K	2.48	129.65	0.05
NORMAL							
8730	257	NET GUN	2/17/94	DUNES	0.19	25.75	0.13
#1		SACRIFICE	12/20/94	OREGON			
#4		SACRIFICE	12/20/94	OREGON			
8490	268	NET GUN	2/19/94	100K	0.1	21.81	0.29
8820	254	NET GUN	2/17/94	DUNES	0.13	16.18	0.52
9920	227	DRIVE NET	3/21/93	N300	0.08	20.45	0.08
#2		SACRIFICE	12/20/94	OREGON			
#3		SACRIFICE	12/20/94	OREGON			
8130	260	NET GUN	2/18/94	100F	0.1	15.08	0.09
*8130		HARVEST	10/19/94	GABLE MT.			
8310	258	NET GUN	2/18/94	100F	0.26	17.48	3.36
8470	267	NET GUN	2/18/94	100F	0.53	18.01	1.08
8580	255	NET GUN	2/17/94	DUNES	0.17	9.46	1.31
8910	256	NET GUN	2/17/94	DUNES	0.4	14.3	0.52
8990	251	NET GUN	2/17/94	DUNES	0.13	99.61	0.26
9860	Y0042	DRIVE NET		N300			
9890	217	DRIVE NET	3/21/93	DUNES	1.99	117.79	0.36
9950	206	DRIVE NET	3/21/93	N300			
9970	219	DRIVE NET	3/21/93	HTS			
8430	250	NET GUN	2/18/94	100F	0.11	17.27	0.15
8970	253	NET GUN	2/17/94	DUNES	0.45	50.08	0.43
8210	264	NET GUN	2/19/94	100D	0.2	16.15	0.1
8710	Y0005	DRIVE NET	2/5/92	100F			
*8710		NET GUN	2/19/94	100H	0.27	18.23	0.15
*8710		DART GUN	12/94	100F			

Appendix F

Contaiminant Levels in Deer

Table F.1. Results of Individual Metal Analyses

METALS IN TISSUE SAMPLES
Deer Liver Analysis
(concentrations in µg/g dry wt - blank corrected)
indicates essentially all results below detection limit

MSL Code	Sponsor Id	Age Sampled	Ag 109 ICP/MS	As75 ICP/MS	Be 9 ICP/MS	Sb 121 ICP/MS	Se 77 GFAA	Tl 205 ICP/MS	Cr 52 ICP/MS	Cd 114 ICP/MS	Cu 63 ICP/MS	Hg CVAA	Ni 62 ICP/MS	Pb 208 ICP/MS	Zn 64 ICP/MS	Fe 57
Atrophy																
871-3	149.920	3	0.112	0.037	0.15	0.02	1.20	0.034	0.18	1.06	62.77	0.0117	0.056	0.075	94.2	na
871-11	Buck X	3	0.133	0.037	0.15	0.02	0.42	0.034	0.07	0.406	10.02	0.0442	0.056	0.026	126.2	na
871-6	149.960	4	0.145	0.037	0.03	0.02	0.71	0.034	0.10	0.329	20.62	0.0083	0.056	0.005	87.3	na
1004-43	148.930	8	0.220	0.830	0.06	0.19	1.31	0.040	0.35	0.865	42.29	0.0010	0.368	0.080	155.7	644.8
1004-42	148.900	12	0.220	0.830	0.06	0.19	1.31	0.040	0.36	0.656	88.30	0.0021	0.770	0.080	115.4	306.2
1004-39	148.090	14	0.220	0.830	0.06	0.19	1.38	0.040	0.47	0.915	112.20	0.0015	1.068	0.080	138.4	912.7
Mean (n = 6)			0.150	0.372	0.07	0.09	0.90	0.032	0.22	0.604	48.03	0.010	0.339	0.049	102.4	266.2
Normal																
1004-34	BUCKY	2	0.220	0.830	0.06	0.19	1.31	0.040	0.32	1.750	124.60	0.0450	0.990	0.080	127.1	698.2
871-8	148.130	3	0.127	0.050	0.04	0.02	0.71	0.014	0.23	0.670	92.42	0.0119	0.056	0.086	876.2	na
1004-45	North#2	4	0.220	0.830	0.06	0.19	1.31	0.040	0.43	0.237	23.89	0.0010	0.250	0.080	156.6	309.1
1004-44	North#1	6	0.220	0.830	0.06	0.19	1.31	0.040	0.39	0.556	29.92	0.0010	0.321	0.080	128.5	614.2
1004-41	148.710	6	0.220	0.830	0.06	0.19	1.31	0.040	0.34	0.674	62.74	0.0035	0.564	0.080	145.3	276.9
1004-40	148.370	9	0.220	0.830	0.06	0.19	1.31	0.040	0.44	0.718	38.90	0.0010	0.302	0.080	183.0	590.4
Mean (n = 6)			0.205	0.700	0.057	0.162	1.21	0.036	0.36	0.767	62.08	0.011	0.414	0.081	269.4	414.8
Oregon																
871-14	OR #1	1	0.113	0.062	0.02	0.02	0.97	0.034	0.07	0.387	155.82	0.0155	0.056	0.037	138.2	na
871-22	OR #4	1	0.130	0.081	0.15	0.02	0.59	0.001	0.11	0.392	160.82	0.0149	0.056	0.257	133.2	na
871-16	OR #2	2	0.182	0.037	0.15	0.02	0.71	0.034	0.07	0.209	134.82	0.0154	0.056	0.007	155.2	na
871-19	OR #3	2	0.146	0.037	0.15	0.02	0.76	0.034	0.07	0.367	122.82	0.0105	0.056	0.026	105.2	na
Mean (n = 4)			0.143	0.054	0.118	0.021	0.76	0.026	0.08	0.339	143.57	0.014	0.056	0.082	132.9	
D.L.			0.220	0.830	0.06	0.19	1.31	0.040	0.08	0.081	1.20	0.0010	0.250	0.080	1.4	3.0
D.L.			0.055	0.037	0.15	0.02	0.19	0.034	0.07	0.081	0.07	0.0011	0.056	0.005	0.3	

Table F.2. Individual Results for Radiological Analyses

Animal ID	Age	^{137}Cs in Muscle[a]	^{90}Sr in Bone[b]	^{90}Sr in An
Atrophy				
148.930	8	0.010	0.39	na
148.900	12	*0.012*	0.42	na
148.090	14	*0.001*	0.49	na
149.920	3	*0.009*	na?	0.24
149.960	4	0.007	0.22	??
RoadKill	10	0.001	0.02	na
148.140	10	*0.007*	*0.00*	na
Buck X	3	na	na	na
148.020	6	na	na	0.13
148.980	12	na	na	0.15
148.250	10	na	na	0.21
Mean (+/- 1 S.E.)	8.4	0.007 (0.002)	0.3 (0.1)	0.2 (0.0
Normal				
148.710	6	*0.025*	0.56	0.28
north#1	6	*0.008*	0.59	na
north#2	4	*0.0003*	0.32	na
148.370	9	*0.001*	0.46	na
148.130	3	0.008	na	na
148.990	2	na	na	0.10
148.970	3	na	na	0.23
148.820	2	na	na	0.23
148.910	1	na	na	0.17
148.580	2	na	na	0.38
148.470	2	na	na	0.57
148.430	3	na	na	0.13
148.310	2	na	na	0.51
148.130	2	na	na	0.16
148.490	1	na	na	0.93
Mean (+/- 1 S.E.)	3.8	0.008 (0.005)	0.48 (0.06)	0.34 (0.
Oregon				
#1	1	-0.004	0.09	na
#2	2	0.013	0.12	na
#3	2	0.034	0.13	na
#4	1	0.010	0.12	na
Reference[d]				2.1 (0.3
Mean (+/- 1 S.E.)	1.5	0.01 (0.008)	0.11 (0.01)	2.1 (0.3

(na) indicates sample was not available

italics indicate results were less than overall analytical counting error.

[a] - pCi/g wet weight muscle tissue.

[b] - pCi/g wet weight bone tissue collected September, 1996.

[c] - pCi/g wet weight antler tissue collected Jan-Feb, 1993-1994.

[d] - Mean value reported from 10 antler samples collected near Silver Lake, OR (see Tiller et al.

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