

Monitoring Reproduction and Contraception in Free Ranging Wildlife: Tule Elk (*Cervus elaphus nannodes*) at Point Reyes National Seashore

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Abstract—The desire of the public to protect free-ranging animals in environments that are devoid of natural regulators can lead to overabundance of urban wildlife in closed habitats. In some parks and reserves, innovative management plans are needed that will include protocols to determine when and if artificial methods of population control should be applied to free-ranging wildlife as an acceptable alternative to hunting or culling. One management option that soon may be available for wildlife application is contraception. Information important to the development of approved contraception programs includes assessment of reproductive characteristics of the targeted species under changing environmental conditions as well as assessment of contraceptive efficacy. This information will improve the ability to accurately estimate the demographic characteristics of animal populations warranting management intervention as well as an evaluation of the success of interventions.

Overabundance of animals associated with increased urbanization of open spaces is a national problem, occurring with increasing frequency in national parks, reserves, national monuments and on private lands (Hadidian 1993; Tzilkowski and others 1993; Supernaugh 1993). Increased contact between the public and wildlife has sharpened public interest in protecting wildlife in environments now devoid of natural controls. As mentioned above, it has been suggested that the absence of controls can lead to overpopulation, destruction of habitat and, ultimately, the potential for population collapse as susceptibility to disease increases with increased numbers (Mc Collough and others 1993).

In national parks, reserves and private lands that are large enough to support ecological process management, artificial intervention is not needed. Ecological process management, as used here, refers to the management of public lands by allowing ecological processes to occur, including, but not limited to periodic disturbances such as fire, floods, drought, and herbivory, predation, births, deaths, decomposition, nutrient cycling, etc., as described by Boyce (1998). In public lands where such management is not an option, some form of intervention to regulate animal population growth is required. Traditional methods of direct intervention consist of culling, hunting and,

where possible, relocation. Indirect intervention, such as the reintroduction of predator species or the removal of available critical resources, is also used. In national parklands like Point Reyes National Seashore (PORE), where much of the land is multiuse, and where an educated and well-funded advocacy constituency is actively involved in all aspects of park management, many of these traditional methods are not acceptable. Frequently, the preferred management option is self-regulation. Most people recognize, however, that self-regulation is not likely where natural regulators have been removed. One approach to animal population control that appeals to the public and which may have use for management is wildlife contraception.

Recent developments in noncapture field methods have improved our ability to contracept wildlife and to evaluate efficacy by monitoring reproductive physiologic patterns in free-ranging wildlife. Current sampling methods decrease risks to animals and researchers, reduce the difficulties of sample collection and storage and increase the amount of sample available for analyses (Kirkpatrick and others 1996b). Specifically, developments in urinary and fecal hormone assay techniques have provided the additional advantage of making it possible to use samples that are adaptable to many different assay formats. This adaptability of the sample to various assay formats allows assay systems to maximize the high hormone concentrations found in excreta and to obtain integrated hormone measures reflecting current reproductive status, as opposed to acute reactions to the stresses of capture and venipuncture. The work presented here uses fecal ovarian steroid monitoring of reproduction to evaluate contraceptive efficacy in free-ranging wildlife, using the California native tule elk (*Cervus elaphus nannodes*) as an example.

The tule elk is a native California deer subspecies that ranged in the hundreds of thousands throughout the state prior to the mid-1800s. By 1873, over-exploitation, habitat reduction and disease reduced their numbers to the point of near extinction. At that time tule elk were accorded full protection under California State law. As a protected species, elk numbers increased from five to ten remaining elk in Kern County, California, to approximately 145 by 1905 (Mc Cullough 1969). From this core group of animals, herds were established in Colusa County and in Owens Valley. In 1974, the California Department of Fish and Game (CDFG) and PORE agreed to begin the reintroduction of the tule elk to a suitable range on the seashore. The physical translocation of 10 elk from the San Luis National Wildlife Refuge to Point Reyes took place in 1978 (Gogan 1986) as part of the CDFG's conservation program and in response to the National Park Service's mandate to restore extirpated forms of wildlife to their historic former ranges. The Tule

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Elk Range at PORE is a 1,050 hectare enclosed area, a former dairy cattle range, located on Tomales Point at the north end of Point Reyes Peninsula in Marin County. On the eastern side of the point are Tomales Bay and the San Andreas Fault Zone, to the west, the Pacific Ocean. The weather is a temperate marine climate with moderate winters, some rain and warm, dry summers, with fog along the coast.

Since the initial introduction in 1978, the elk have reproduced without unnatural restriction. While the population growth rate was slow over the first 12 years, it has steadily increased over the last five years. An initial carrying capacity of between 140 and 350 animals was estimated for this recovering habitat in 1986 (Gogan 1986). As of 1997, 465 tule elk were on the Tomales Point Elk Range. A revised, more recently established carrying capacity for the elk range was set at 330 (Bartolome 1993). Currently, in 1999, there are approximately 552 elk on the Tomales Point Elk Range, with an anticipated 70-100 calves due prior to the 1999 rutting season. It is uncertain whether this current growth rate, if sustained, will precipitate the degradation of the habitat or have other negative impacts on sensitive plant and animal species also located on the Tomales Point Elk Range, as suggested earlier (Mc Collough and others 1993).

In the mild habitat of the Tomales Point Elk Range where most natural regulators have been removed—with year-round food and water availability, no possible dispersion or emigration and no large predator populations—there is an apparent need for management to slow the rate of increase of elk to prevent overpopulation. In this study, we tested the efficacy of the double shot pZP vaccine in the tule elk. This vaccine is administered once by hand-injection to elk cows at the time of capture and radio collaring and by giving a remote booster shot by dart gun three to five weeks later. The principal objective of this study was to assist PORE in assessing its options for elk population control by developing the technology to predict realized reproductive output and to control increases in population numbers in a closed habitat. Fecal ovarian steroid hormones were monitored to assess the reproductive condition of untreated and treated elk cows and to monitor the efficacy of the contraceptive agent in cows treated with the immuno-contraceptive “pZP” (porcine zona pellucida).

Materials and Methods

Field Methods

Sixty-four radio-collared adult elk cows were included in this study. Twenty-nine of these cows were treated with pZP vaccine (obtained through the Humane Society of the United States; manufactured by the laboratory of Dr. Irwin Liu, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California) to inhibit reproduction in early July of 1997. An initial injection of emulsified immunogen (500ul of 135ug pZP in 500ul Freund's Complete Adjuvant™) was administered by hand at the time of capture and collaring. Three to five weeks later, in August, these same cows were given a booster shot (500ul of 135ug pZP emulsified in 500ul Freund's Incomplete Adjuvant™) by remotely delivered darts, using a Model 193 cartridge-fired rifle (Pneu

Dart, Inc., Williamsport, PA). The remaining 35 adult radio-collared elk cows were untreated.

Following immunization, all collared animals were located twice monthly and observed at distances of 15 to 400 yards with binoculars and a sighting scope. Fecal samples were collected on all radio-collared females and on uncollared females for ovarian steroid hormone measurements. Details of the methods involved in the sample collection and laboratory analyses of these samples have been published elsewhere (Stoops and others 1999). Briefly, when individually identified animals defecated, the location of the feces was noted and a range finder was used to determine the distance to the animal from the point of observation. One person on a team used a hand-held radio to direct another to collect fecal samples, using laboratory gloves and plastic zip top bags. In general, more than one fecal sample was spotted for a particular group of elk cows. Time and distance were recorded, and location maps were drawn for each sample in order to maximize the number of samples collected per group. Fecal samples were also collected from specifically identified uncollared elk cows that were distinguished from collared cows or spike bulls. At least 5.0-g of fecal matter was collected and placed in appropriately labeled plastic bags. Samples were frozen within three to six hours of collection without preservatives until laboratory analyses.

Written records were kept for each group of elk encountered while hiking or riding the elk range. These records consisted of the location of the sighting, the time of the sighting, the total number of animals, the number of spike bulls, sub-prime branched bulls, branched bulls, cows and calves and a listing of all sighted radio-collars in the group. Continued attention to a radio-collared cow from prime and/or sub-prime bull past the time of the rut was recorded, and the cow involved was identified. The collection of additional demographic data during the calving season included calf confirmations determined by visual observance of nursing. All mortalities observed were flagged and their locations were recorded. Radio-collars and ear tags were retrieved, if applicable.

Laboratory Methods

Fecal samples were logged into laboratory records on a computerized database. The identification code of the female elk and the date and location of collection were put onto a spreadsheet; hormone concentrations and conversion factors were added after assaying. Samples were thawed, and five grams of fecal material were placed into individual glass scintillation vials. The uncapped vials were then put into a drying oven, where they remained for 72 hours until totally dry. Dried fecal material was chopped into small fragments and powder. A dry weight of .250 grams fecal material was placed in a 16-x 100-mm glass test tube with 30 parts of 40% MeOH 60% EIA wash solubilizing solution, bringing the total weight of the dry feces and the solubilizer to 7.75 grams. Tubes were capped and shaken for 48 hours at room temperature. Tubes were next placed in a refrigerated centrifuge and spun at 5,000 rpms for 20 minutes. The supernatant was decanted into 12x75mm plastic tubes, and diluted with distilled water to the appropriate concentrations for each assay. Specific details of validation and application of enzyme immunoassays to tule elk fecal samples have been

described elsewhere (Stoops and others 1999). Estrogen and progesterone fecal metabolite profiles were used to identify cyclicity, conception and pregnancy loss. They were also used to anticipate parturition in the study animals, as well as to make comparisons between treated and untreated elk cows.

Statistical Methods

Standard descriptive statistics were used to summarize results. F Tests were performed to test whether the treated, nonpregnant and the untreated, pregnant elk cows had the same or equal variances in hormone excretion over the months studied.

Results

Of the 29 elk cows treated with pZP prior to the rut in 1997, 22 had calves from the previous year's rut. This represents 79% fertility for the treated cows in 1996. There is no reason to assume that this percentage is atypical or that it skewed the contraception result obtained, since 24, or 71%, of the 34 untreated radio-collared elk cows calved in consecutive years. In fact, six, or 43%, of the 14 remaining originally radio-collared animals from 1995 had calves for at least three consecutive years. Furthermore, a total of 11 elk cows, or 31% of the total untreated radio-collared population (n = 35), had calves for four consecutive years.

After treatment in the summer of 1997, and after calving in the spring and early summer of 1998, 77% of the untreated radio-collared cows calved compared to 7% of the treated radio-collared cows. Stated in reverse, 93% of the treated cows were infertile, compared with 23% of the untreated cows. Untreated cows included one-year and two-year old cows, which do not characteristically have calves at Point Reyes.

Based on fecal steroid evaluations, tables 1 and 2 show the percentages of untreated and treated cows sampled each

month that were cycling or pregnant from May 1997 through April 1998. Stoops and others (1999) reported that steroid hormone metabolite levels prior to December could not be used to distinguish between cycling and early pregnant elk cows. This observation, paired with the additional observation that progesterone metabolite hormone levels of >1.01 µg/gr dwf (dry weight feces) from the month of December onward were 100% effective in diagnosing pregnancy in untreated radio-collared tule elk cows, were used to designate cows as either cycling or pregnant in these tables. Both groups (treated and untreated) show a high percentage of either cycling or pregnant elk cows, with the lowest percentage occurring in December for both groups. It is likely that the progesterone metabolite values of >1.01 µg/gr dwf in untreated elk cows, however, reflect pregnancy, whereas elevated values in the treated cows reflect ongoing cyclicity.

The annual endocrine profiles of the ovarian fecal steroid metabolites are depicted in figures 1-2. Figure 1 illustrates differences in the annual mean, unaligned estrogen metabolite levels of pregnant, untreated elk cows and treated, nonpregnant cows. In the untreated cows, mean, unaligned estrogen metabolite levels increase from 68.73 ± 43.52 (SD) ng/gr dwf in July to 101.56 ± 49.03 (SD) ng/gr dwf in November. Estrogen levels drop to lowest levels, 38.81 ± 11.77 (SD) ng/gr dwf in January, before beginning their pre-parturient climb toward peak values, 353.94 ± 337.46 (SD) ng/gr dwf in April. The estrogen profile for nonpregnant, treated, and therefore presumably continuously cycling, radio-collared elk cows has a similar pattern, but lower overall absolute levels, with a November high of 88.39 ± 52.67 (SD) ng/gr dwf, a January low of 35.88 ± 16.92 (SD) and an April climb to only 138.06 ± 196.51 (SD) ng/gr dwf. Figure 2 depicts differences between in the two groups with respect to mean, unaligned progesterone metabolite levels. In the untreated cows, progesterone metabolite levels increase from 1.81 ± 0.73 (SD) µg/gr dwf in July to 3.48 ± 2.15 (SD) µg/gr dwf in November. Progesterone metabolite levels then began their pre-parturient climb toward peak values, 4.89 ± 2.06 (SD) µg/gr dwf in May, falling only slightly in April to

Table 1—Estimated percentages of untreated radio-collared adult tule elk cows^a cycling or pregnant and average ovarian fecal steroid hormone metabolite levels.

| Month | N | % | Monthly average fecal estrogen metabolite levels, unaligned ng/gr. dwf ^b (±SD) | Monthly average progesterone metabolite levels, unaligned µg/gr. dwf (±SD) |
|----------------|-----|--------|---|--|
| May 1997 | 10 | 90.00 | 67.19 ± 22.54 | 1.45 ± 0.28 |
| June 1997 | 15 | 100.00 | 48.71 ± 27.37 | 2.22 ± 0.54 |
| July 1997 | 32 | 97.73 | 67.68 ± 48.28 | 1.99 ± 0.33 |
| August 1997 | 18 | 67.00 | 92.98 ± 29.43 | 2.55 ± 0.96 |
| September 1997 | 70 | 78.70 | 96.79 ± 33.78 | 2.71 ± 1.31 |
| October 1997 | 120 | 83.85 | 73.34 ± 42.59 | 2.23 ± 0.80 |
| November 1997 | 76 | 81.46 | 101.93 ± 45.54 | 2.64 ± 1.69 |
| December 1997 | 64 | 54.68 | 82.17 ± 41.25 | 2.16 ± 1.48 |
| January 1998 | 59 | 61.02 | 57.41 ± 21.15 | 2.88 ± 1.66 |
| February 1998 | 44 | 84.60 | 80.38 ± 20.89 | 3.21 ± 1.73 |
| March 1998 | 100 | 78.28 | 124.34 ± 95.77 | 3.95 ± 2.24 |
| April 1998 | 45 | 82.22 | 267.49 ± 421.25 | 3.90 ± 2.51 |

^an = 95 (estimated effective breeding population of elk cows = 125 (Howell, pers. comm), minus the treated radio-collared group of 30 cows).

^bdwf = dry weight feces.

Table 2—Estimated percentages of treated radio-collared adult tule elk cows^a cycling or pregnant and average ovarian fecal steroid hormone metabolite levels.

| Month | N | % | Monthly average fecal estrogen metabolite levels, unaligned ng/gr. dwf ^b (±SD) | Monthly average progesterone metabolite levels, unaligned µg/gr. dwf (±SD) |
|----------------|----|-------|---|--|
| May 1997 | | | | |
| June 1997 | | | | |
| July 1997 | | | | |
| August 1997 | 29 | 79.31 | 49.30 ± 21.92 | 1.80 ± 0.82 |
| September 1997 | 24 | 45.83 | 76.65 ± 21.67 | 2.47 ± 0.90 |
| October 1997 | 28 | 81.25 | 73.59 ± 25.62 | 2.17 ± 1.04 |
| November 1997 | 20 | 65.27 | 97.21 ± 58.50 | 2.64 ± 1.82 |
| December 1997 | 21 | 35.10 | 96.98 ± 37.57 | 2.83 ± 2.69 |
| January 1998 | 18 | 74.28 | 36.59 ± 18.02 | 2.64 ± 1.82 |
| February 1998 | 12 | 66.67 | 80.93 ± 40.32 | 4.13 ± 1.71 |
| March 1998 | 15 | 81.25 | 70.77 ± 15.43 | 2.94 ± 1.33 |
| April 1998 | 28 | 71.30 | 237.00 ± 195.75 | 3.13 ± 1.90 |

^an = 28 (from an “n” of 30, excluding one cow that was not treated and one cow that was pregnant at the time of treatment and including two cows that conceived after treatment).

^bdwf = dry weight feces.

4.20 ± 2.26 (SD) µg/gr dwf. The profile for nonpregnant, treated, radio-collared elk cows had a different pattern and different lower overall absolute levels, with a November high of 1.85 ± 1.92 (SD) ng/gr dwf, a February peak of 3.01 ± 2.23 (SD) and a March-April fall to 1.48 ± 3.89 (SD) µg/gr dwf and 1.71 ± 4.15 (SD) µg/gr dwf, respectively.

F Tests for comparison of hormone metabolites in treated, nonpregnant elk cows (n = 26) to those in untreated, pregnant elk cows (n = 27) over the period studied indicated a statistically significant difference between the two groups with respect to estrogen metabolite excretion over the year

(p > 0.00), but none for progesterone metabolite excretion (p > 0.07).

Discussion

One of the consequences of the “Greening of America” and increased urbanization is an overabundance of certain wildlife populations such as deer, elk, mountain goats, skunks, and raccoons. The management of these wildlife populations is a major problem facing wildlife professionals that will not

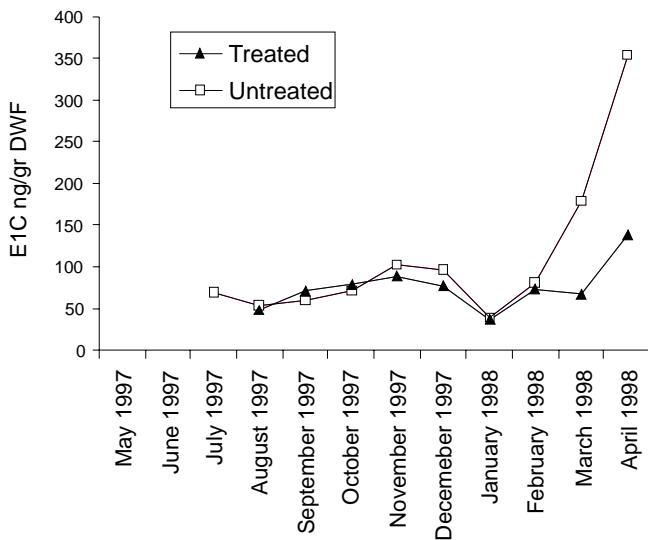


Figure 1—The annual endocrine profiles of annual mean, unaligned estrogen metabolites levels of pregnant, untreated elk cows and treated, nonpregnant cows. F Tests for comparison of fecal estrogen in treated, non-pregnant cows (n = 26) to those in untreated, pregnant cows (n = 27) over the period studied indicated a statistically significant difference between the two groups with respect to estrogen metabolite excretion over the year (p > 0.001).

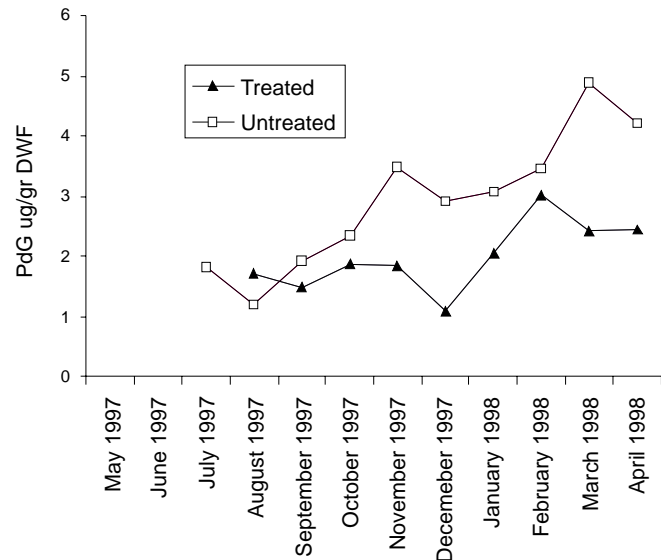


Figure 2—The annual endocrine profiles of annual mean, unaligned progesterone metabolites levels of pregnant, untreated elk cows and treated, nonpregnant cows. F Tests for comparison of fecal estrogen in treated, nonpregnant cows (n = 26) to those in untreated, pregnant cows (n = 27) over the period studied indicated no statistically significant difference between the two groups with respect to estrogen metabolite excretion over the year (p > 0.07).

be resolved easily. The dimensionality of the problem is complex, but not in terms of the biology or ecology of species involved; rather, the complexity stems from social, political, legal and economic concerns. For example, in the case of overly abundant white-tailed and mule deer, described by Conover (1997), these animals cause an estimated annual loss of two billion dollars (in car, crop, timber and household damage), but they have a recreational value of more than 12 billion dollars after subtracting losses. In addition, the presence of these deer in urbanized open spaces provide intangible but publicly recognized values, such as a general sense of well being and aesthetic pleasure, as well as ecological or biologic values like deer-induced alternate stable habitats (Conover 1997). Local experiences with the management of deer overabundance throughout the eastern United States suggest that solutions to urban wildlife overpopulation will require the mutual understanding and agreement of both the public and agencies involved on management goals and values to be preserved wherever intervention is needed.

While contraception cannot reverse population growth rates, it can slow them. Several credible programs have been initiated to develop artificial methods for population control, and some of these methods, such as immunocontraception, have been shown to be apparently safe and effective (Kirkpatrick 1990, 1991, 1994, 1995; Kirkpatrick and others 1995a, 1995b, 1997; Plotka and Seal 1989; Turner and others 1992, 1996; Warren and others 1992). The pZP (porcine zona pellucida) vaccine has been shown to be an effective immuno-contraceptive in Roosevelt elk and Rocky Mountain elk (Kirkpatrick and others 1996; Garrot and others 1998), but had not been used in tule elk prior to the use reported here. Using contraception to slow growth rates allows additional time for the further development of publicly acceptable management options that can reverse the growth rates, such as immuno-sterilization.

The results presented here on the California tule elk confirm that pZP vaccination is an effective contraception method for elk cows. Two of the treated cows were either pregnant before titers were high enough to prevent conception or became pregnant after treatment. These two cows received their initial immunization in early August and their boosters in the second week of September 1997. They both had live calves in late June and early July 1998, suggesting conception dates in early to mid-October. It is possible that a month—between the time of the second immunization and the approximate time of conception—was not long enough for the cows to mount an antibody response sufficient to prevent conception. Another alternative is that the amount of immunogen presumed to be delivered by remote darting was not delivered, and the dosage was, therefore, suboptimal. Nonetheless, pZP reduced the rate of calving in the treated group to >70% of that of the untreated group. In addition, according to J. A. Howell (1998), the rate of population increase in the Tomales Point elk herd in 1996-97 was approximately 32%. His population count in 1997-98 was 470 animals, an estimated rate of population increase of *ca.* 26% for that year. This is not a statistically significant reduction in the rate of increase, but it does coincide with the number of animals treated, suggesting that increasing the number of contracepted elk cows will continue slow reproduction and reduce this rate.

At the start of the rut in 1999, only 15% of the 13 initially treated elk cows from 1997 not given booster shots by dart gun in the summer of 1998 had calved by the summer of 1999 (2 out of 13 cows). This suggests the possibility that for perhaps as many as 85% of elk cows, annual booster shots may not be required. Additional booster shots may only be required every second or even every third year to maintain contraception. Furthermore, work in progress suggests that when pZP is presented to the elk cow immune system, depending on the formulation of the presentation, cows may be contracepted or sterilized with an initial immunization. These findings have useful implications for management. Single shot formulations and decreased immunization frequency will reduce the cost involved in contraception programs using pZP. Having a sterilization option provides an acceptable means of animal removal and of slowing the rate of increase while keeping resources occupied rather than freeing them for another animals' reproductive gain.

As stated earlier, these tools are especially valuable to private and public parks and recreation areas where traditional management tools can not be employed and where management's wildlife ethic is constrained by public opinion. The use of immuno-contraception with population monitoring of ovarian hormones to estimate fecundity in a given habitat, provides a lead-time to implement options for the next breeding season: maximal yield for a given population is predictable before calving takes place on the basis of fecundity estimates. The option presented here, fecal hormone monitoring and pZP immuno-contraception, together with work in progress on single-shot formulations of pZP contraceptive and sterilant, furnishes management with a few publicly acceptable tools to assist in animal population regulation required by increasingly common conditions of urbanization.

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