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Original Article

Using Fecal Progestagens and Logistic Regression to Enhance Pregnancy Detection in Wild Ungulates: A Bison Case Study

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ABSTRACT Ungulate ecological studies often include components of reproduction because of its demographic importance and the ecological factors affecting it. Pregnancy status, in particular, is key because it represents a starting point for succeeding measurements of vital rates. Here, we present a case study using wild bison (*Bison bison*), in which we developed a non-invasive method for assessing pregnancy in unmarked, non-handled animals that improves upon existing approaches for wild ungulates. Specifically, we employed a model-based binary logistic-regression approach to estimate the probability of pregnancy predicted by fecal progestagen concentrations quantified from a single, late-gestation scat sample. For 155 observations of 42 marked bison from the Jackson herd in northwest Wyoming, USA during 1997–2005, we used combinations of transrectal uterine palpation and calf status as independent measures of pregnancy to reduce the potential for error inherent in using either measure alone. We evaluated predictive success by calculating misprediction rates from leave-one-out cross-validation, and by calculating the percentage of 95% confidence intervals that crossed a pregnant–not-pregnant threshold. Correct predictions, with high confidence, were obtained from a model using year-centered, natural-log-transformed progestagen concentrations, resulting in an overall successful cross-validation pregnancy prediction rate of 93.5%. Our approach will allow practitioners to consider the uncertainty associated with each prediction, thereby improving prediction interpretations. The approach should appeal to practitioners because fecal samples are easily collected and preserved, laboratory procedures are well-documented, and logistic-regression statistical software is readily available. Furthermore, samples can be obtained non-invasively, which reduces cost and potential bias and increases animal safety, human safety, and social acceptability.

KEY WORDS bison, fecal, hormones, logistic, non-invasive, pregnancy, pregnancy-specific protein B, regression, Wyoming, Yellowstone.

Studies of ungulates often include components of reproduction because of its importance to demography (e.g., Testa 2004, Coulson et al. 2005, Fuller et al. 2007) and the ecological factors affecting it (Berger et al. 1999). Among parameters commonly used to assess reproductive performance, pregnancy status is key because, following ovulation, it represents a starting point for succeeding measurements of vital rates, including birth rates, neonatal survival, and recruitment of breeding-aged individuals into a population (Ramsay and Sadleir 1979, Messier et al. 1990, Kirkpatrick et al. 1993, Russell et al. 1998, Stoops et al. 1999). Knowledge of pregnancy status can be useful at the population level, where it may be expressed as an overall rate (Messier et al. 1990), or at the individual level, where the status of a marked individual and its subsequent reproductive outcome is of interest (Messier et al. 1990, White et al. 1995, Garrott et al. 1998).

Determining pregnancy of wild ungulates is inherently difficult and presents a serious challenge to researchers interested in reproductive ecology. Handling animals, either through capture and physical restraint or chemical immobilization, is required for direct evaluations of pregnancy status, including transrectal palpation (Hein et al. 1991) and ultrasound (Barrett 1981, Stephenson et al. 1995), as well as for indirect assessments using serum pregnancy-specific protein B (Houston et al. 1986, Sasser et al. 1986, Wood et al. 1986, Haigh et al. 1988). An obvious disadvantage with these methods is that there are hazards associated with handling

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animals, both to the research subjects (e.g., Ballard and Tobey 1981, Valkenburg et al. 1983, Larsen and Gauthier 1989, Peterson et al. 2003) and/or to the handlers (Jessup et al. 1988, Haigh 1990, Dein et al. 2005, Roffe et al. 2005, and references therein), some of which may bias results of the intended research (Welsh and Johnson 1981). The potential for capture-related mortality is of biological concern as well, particularly when handling rare or endangered species (Association for the Study of Animal Behaviour 2003, Berger et al. 2010). Likewise, society increasingly questions (on ethical grounds) the need to manipulate animals or employ invasive, potentially harmful handling techniques (Farnsworth and Rosovsky 1993, see Bekoff and Jamieson 1996, Peterson et al. 2003).

In general, non-invasive pregnancy assessment methods (Lasley and Kirkpatrick 1991, Monfort 2003) are more limited in inferential capability but have several inherent advantages, including 1) safety, because capture and handling are not required; 2) reduced bias from potential effects on behavior, activity, distribution, or physiology; 3) lower expense because immobilizing drugs or specially trained personnel to administer them or to perform uterine transrectal palpation are not necessary; and 4) social acceptability. In ungulates, these techniques have focused on the measurement of reproductive steroid hormone metabolites excreted in urine (Poole et al. 1984, Kirkpatrick et al. 1990, Monfort et al. 1990) and/or feces (Kirkpatrick et al. 1990, Monfort et al. 1993, Garrott et al. 1998, Berger et al. 1999, Schoenecker et al. 2004). Application of these techniques to accurately estimate pregnancy status for individual animals from single fecal samples, which is more cost-efficient and less invasive than collecting multiple samples per individual, has been proposed only for elk (*Cervus elaphus*; Garrott et al. 1998), bighorn sheep (*Ovis canadensis*; Schoenecker et al. 2004), and feral horses (Linklater et al. 2000).

Bison (*Bison bison*) are polygynous seasonal breeders with a 9-month gestation period (Berger and Cunningham 1994). In our study area, breeding and calving peaked in August and May, respectively, with most births occurring over a 2-month period (Berger and Cain 1999).

As part of broader research on bison reproductive ecology, we sought to evaluate fecundity and prevalence of fetal and neonatal loss in a marked sample of reproductive-aged animals. We directly assessed pregnancy status in a subset of our bison population that was handled each year, with the primary intent of determining the incidence of brucellosis and its effect on reproduction. Our goal in the current study was to develop a non-invasive method for assessing pregnancy status for the remainder of our study animals that were not handled each year. Reproductive-aged females were observed directly at least once weekly during the presumptive parturition interval to estimate date of birth. Our study population was wide-ranging and this was a labor-intensive task, so our objective was to improve efficiency by removing animals that we were confident were not pregnant from the group checked for calves each week.

Our study area did not have continuous snow cover in winter, so urine collection was not feasible. Therefore, we focused on developing model-based estimates of pregnancy probability based on quantitative assessments of fecal progestagens in a single scat collected during winter. We recognized the limitations of using single samples, but, similar to many studies, cost, logistical considerations, and the potential for additional disturbance to our study population precluded additional fecal-sample collections. Based on past research (Kirkpatrick et al. 1992, 1993), we expected concentrations of fecal progestagens to differ between pregnant and non-pregnant bison. However, in contrast to similar ungulate studies, our goal was not to simply compare mean concentrations of fecal progestagens between pregnant and non-pregnant groups, but instead to estimate the probability of pregnancy for individual females based on hormone concentration, and then to use this probability to predict pregnancy status. By quantifying the uncertainty inherent in our probability estimates, we were also able gauge confidence in our predictive measures.

STUDY AREA

Our study area in the southern Greater Yellowstone Ecosystem, USA, focused on Grand Teton National Park and the National Elk Refuge, which comprised the primary range of the free-ranging Jackson bison population. This area included the upper Snake River drainage in a high-elevation valley commonly referred to as Jackson Hole, bounded by the Teton Range to the west, the Gros Ventre and Absaroka Mountains to the east, the Yellowstone Plateau to the north, and the town of Jackson, Wyoming to the south. During our 1997–2005 study, the population varied from 320 to 950 (S. L. Cain, unpublished data). Pelleted alfalfa was provided to elk and bison on the National Elk Refuge for an average of 57 days (range = 28–85) each winter (E. Cole, National Elk Refuge, unpublished data).

Brucellosis was discovered in the Jackson bison herd in 1989 (Williams et al. 1993). Its source has been attributed to the original Yellowstone reintroduction because these bison were known to be infected (Meagher 1973), or to sympatric elk from the Jackson elk herd, which were also known to be infected (Thorne 1982). Based on a criterion that required a minimum of 2 out of 6 serologic tests to be positive by bovine Uniform Methods and Rules (USDA APHIS 2003; at least one of which had to be a quantitative test), brucellosis annual seroprevalence in non-calf, predominantly female, bison averaged 69% during our study (T. J. Roffe, unpublished data). We considered bison sampled more than once during the year as positive if they met the criteria for positive on any sample.

METHODS

Sample Collection and Processing

Our study protocol required marking, taking samples from, and monitoring the reproductive performance of a sample of female bison each year. We immobilized bison on or near National Elk Refuge feed-lines mid-winter (late Jan–mid-Mar), during the 2nd or early 3rd pregnancy trimester, from a tracked snow-vehicle using a carfentanil–xylazine drug com-

bination administered from a projectile dart (Roffe et al. 2001). We deployed Telonics very-high-frequency radiocollars (Telonics, Inc., Mesa, AZ) and numbered 5.8-cm × 7.3-cm bangle ear tags on each individual; individuals were initially selected randomly from groups of several hundred bison on feed-lines. We did this by generating random numbers from 1 to 20 to determine the *n*th adult female we would choose as we approached or worked our way through a group of bison. After marking our original sample in 1997, during 1998–2002 we attempted to re-capture each individual annually to determine pregnancy status, take blood and fecal samples, and replace aged radiocollars. During 2003–2005 we re-captured only half of our marked sample annually to address a separate objective of evaluating the effects of handling on reproduction. We enhanced our sample annually using randomly selected females of appropriate age to replace those that died or whose radiocollars needed replacement.

For each anesthetized bison, transrectal palpation was performed by a veterinarian to assess pregnancy status, 20 cm^3 of whole blood was taken to evaluate brucellosis exposure and pregnancy-specific protein B, approximately 20–50 g of feces was obtained (unless the animal's colon was void), fat depth over the posterior-most rib was measured as an index of body condition (e.g., Richards et al. 1986, McLaren et al. 1991), and tooth eruption patterns were recorded for aging (e.g., Frison and Reher 1970). For animals not immobilized, we obtained fecal samples by locating individuals with radiotelemetry, verifying their identity with ear tags when multiple radioed animals were in a group, watching them until they defecated, and then collecting the target sample. We minimized potential for sampling non-target fecal matter by approaching bison closely, working in pairs whenever possible (allowing one person to remain focused on the target sample while the other approached and collected), using a stationary spotting scope focused on the target sample to corroborate location, sampling only fresh fecal matter, and rejecting samples that we could not confidently attribute to the target animal. We stored fecal samples frozen in sealed plastic bags until they were thawed for laboratory analyses.

Endocrine assessments were conducted at the Smithsonian Conservation Biology Institute (Front Royal, VA). All fecal samples were processed and extracted using fecal hormone procedures that have been previously described (Garrott et al. 1998). Fecal progestagens were quantified with a monoclonal antibody (CL425; C. Munro, University of California–Davis, Davis, CA) used in a radioimmunoassay (1997–2000; Wasser et al. 1994) or enzyme-immunoassay (2001–2005; Graham et al. 2001). For both assays, serially diluted fecal extracts demonstrated displacement curves that were parallel to those of standard hormone preparations. All samples for each year were evaluated in a single assay, and intra-assay variation was $<5\%$.

We centrifuged blood, harvested serum, and froze all samples until processing. Sera were submitted to the American Association of Veterinary Diagnostic Laboratories certified Montana Veterinary Diagnostic Laboratory, Bozeman, Montana (USA), for 8 *Brucella* serologic tests: card (Rose Bengal), buffered antigen plate agglutination, standard plate agglutination, standard tube agglutination, rivanol, and complement fixation. Samples from 2002 to 2005 were also processed using fluorescent polarization. We interpreted tests as positive or negative based on U.S. Department of Agriculture bovine brucellosis uniform methods and rules (USDA APHIS 2003). We classified high-titered animals based on complement fixation of a 4+ reaction at 1:80 dilution or greater (Roffe et al. 1999).

We also submitted sera to Biotracking, Inc. (Moscow, ID; biotracking.com) to determine concentrations of pregnancy-specific protein B (Sasser et al. 1986). Pregnancy-specific protein B was determined by radioimmunoassay (earlier samples) and enzyme-linked immunoassay (later samples) with a reported 95% accuracy at 40 days pregnant in bison (Biotracking, Inc.).

Reproductive Status

To evaluate the relationship between fecal progestagens and pregnancy, we needed an independent measure of pregnancy against which to assess fecal progestagen levels. We defined "pregnant" as all adult females observed with a calf in the spring or summer following winter fecal sampling. We determined "females with calves" by locating each marked reproductive-aged bison at least once weekly during the calving season (Berger and Cain 1999), and assigning calves to specific females only after a suckling was observed (Berger and Cunningham 1994).

We defined as "non-pregnant" those females that were both negative by palpation and were without a calf, because "absence of a calf" alone had a greater margin of error for labeling pregnant females that lost calves through fetal or neonatal mortality as non-pregnant. We eliminated females of uncertain pregnancy status (positive palpation, absence of calf) from our analysis because of the small potential for error by palpation. We recognized that using these criteria incurred a small potential to include females palpated falsely as not-pregnant that subsequently lost their fetus or neonate (and, therefore, were absent calf) in the "not-pregnant" group.

Modeling Procedures and Considerations

We modeled the relationship between pregnancy status and winter-collected (late gestation) fecal progestagens (WP) using binary logistic regression (e.g., Hosmer and Lemeshow 2000). Logistic regression modeled the natural log of the odds [$\ln(\text{odds}) = \text{logit}(P)$] of being pregnant as a linear function of the explanatory variable WP. The odds of being pregnant were defined as the probability of being pregnant, divided by the probability of not being pregnant. For each female, the logistic-regression model provided an estimated logit of the probability of pregnancy, $\ln[P/(1 - P)]$, along with standard errors. We then obtained estimated probabilities of pregnancy simply by inverting the logit transformation. Likewise, we calculated confidence intervals (CI) for probabilities by applying the inverse transformation to the endpoints of the CI calculated on the logit scale (Agresti 2002). R statistical software was used for all analyses (R Development Core Team 2012).

Because our goal was to predict pregnancy status for non-handled females, we focused our model-assessment efforts on quantifying predictive success. Classification of a female as pregnant or not-pregnant based on the magnitude of her WP required specification of a threshold probability. We chose the classification threshold minimizing the mis-prediction rate, as calculated using leave-one-out cross validation. That is, we removed one observation from the data set, fit the model, and then predicted pregnancy status for that observation, which allowed for comparison of predicted status to true status. Repeating this for all observations allowed us to calculate mis-prediction rate as the number of incorrect predictions divided by the total number of observations. We also calculated an average estimation error using cross-validation by taking the absolute value of the difference between the estimated probability of pregnancy and the true response (1 for pregnant, 0 for not pregnant), averaged over all observations. For example, an estimated probability of 0.78 gave an estimation error of 0.22 if the female was pregnant and 0.78 if the female was not pregnant.

The use of mis-prediction rate and average estimation error relied only on the point estimates of the probabilities and the chosen classification threshold, and failed to account for widths of associated CIs for the probabilities. To assess this uncertainty in the context of prediction, we evaluated whether CIs crossed the classification threshold, coupled with whether the pregnancy status was correctly predicted. For correct predictions, it is undesirable for the CI to cross the threshold, which indicates a lack of confidence in a correct prediction. On the other hand, for incorrect predictions, it is undesirable for the CI to *not* cross the threshold, which indicates high confidence in a wrong prediction. In these 2 cases, simply relying on the mis-prediction rate would yield a falsely optimistic or pessimistic view, respectively. We considered these bad predictive behaviors for a model, and calculated the proportion of 95% CIs in each case for a particular model, which allowed comparison of these proportions across models with different covariates. Thus, we evaluated predictive ability by comparing predictions to known values, and utilizing the information in the CI regarding uncertainty in the estimate used to make the prediction.

We considered several issues related to assumptions in our statistical approach. First, the binary logistic-regression model assumed 1) independent observations, and 2) a linear relationship between the log odds of pregnancy and the explanatory variables. Data were collected across years; therefore, we assessed the appropriateness of presumptive homogeneity in the relationship between WP and the probability of pregnancy across years. Initially, we found clear year-to-year differences in the range of WP (Fig. 1A). Factors that can modulate year-to-year variability in fecal steroid excretion include age (Morden et al. 2011) and nutritional plane (Cook et al. 2001, 2002), but we found no relationship between age or body condition and WP for reproductive-aged animals. Nearly all of our bison received supplemental feed (on the National Elk Refuge) at the time of sampling, so large variations in dietary fiber (which may also affect hormone secretion; Wasser et al. 1993), were unlikely. A more likely source of between-year variation in progestagen concentrations related to sampling females at different stages of gestation, which resulted from sampling periods that varied by several weeks among years, or from natural variation in timing of reproductive season onset (Monfort et al. 1993, Garrott et al. 1998), as evidenced by a wide range of within-year calf birth dates in our population (Berger and Cain 1999). However, in addition to sampling strategies and physiology, intra- and inter-assay variation in hormone tests can contribute to the year-to-year variation (see reviews, Monfort 2003, Schwartz and Monfort 2008).

We corrected for year-to-year variation, regardless of its source, by first applying a natural-log transformation to WP, which served to make the variance more constant across years. To adjust for remaining differences in the centers of the distributions across years, we performed a year-centering transformation by subtracting each observation from its

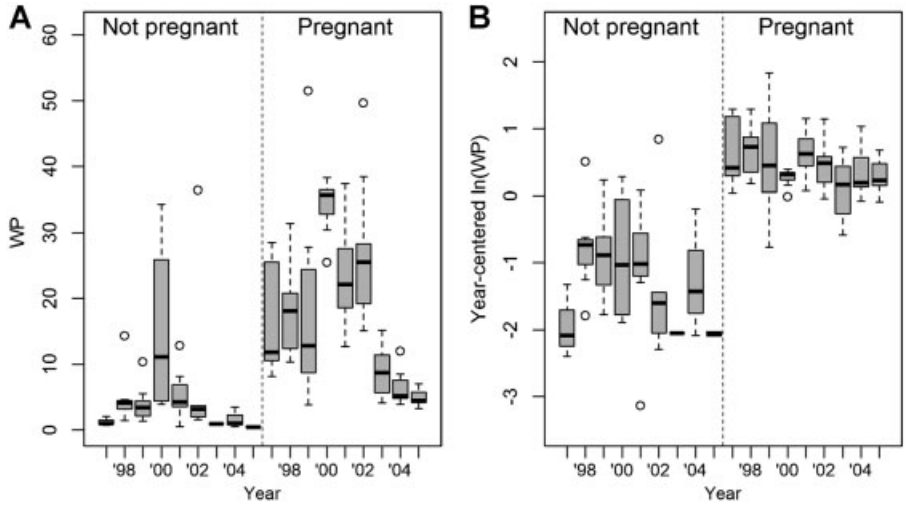

Figure 1. Winter-collected (late gestation) fecal progestagen levels (WP) (A), and year-centered ln(WP) (B) by year and assigned pregnancy status for Jackson bison, northwest Wyoming, USA, 1997–2005 (dark horizontal lines in each box represent medians, boxes span the interquartile range [IQR], whiskers extend to min. and max. observations within 1.5× the IQR, and points denote observations >1.5× the IQR).

associated year average (Fig. 1B). This approach removed most of the dependence of WP on year (Figs. 1 and 2).

Second, because most females were accompanied by multiple observations over time (repeated measures), we assessed the assumption of independence among all observations. Ignoring females in the analysis assumed the relationship between the probability of pregnancy and (transformed) WP was the same for each female (homogeneity among F), and that each observation was independent, even if it came from the same female in a different year. However, we found no clear indication of heterogeneity among females. For example, a visual cut-off of approximately zero for year-centered ln(WP) was effective at separating pregnant from non-pregnant observations, regardless of the female or year (Fig. 2). We decided against using random effects for females because of the small number of repeated measures for most individuals and our ultimate interest in predicting for females outside the sample. However, we did use generalized estimating equations to compare an "independent" model to one accounting for within-female dependence (e.g., Zurr et al. 2009) and detected no practical difference in the estimates or standard errors between the models.

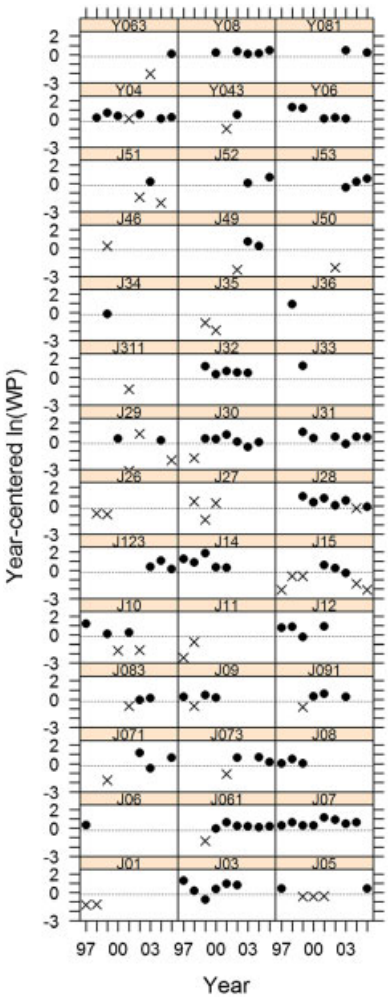

Figure 2. Year-centered, log-transformed values of winter-collected (late gestation) fecal progestagens (WP) by year and assigned pregnancy status (solid circle = pregnant, X = not pregnant) for 42 female bison from the Jackson bison herd, northwest Wyoming, USA, 1997–2005.

Third, we assessed the potential for serial dependence among the binary responses from the same female, which would manifest as longer successive sequences (runs) of 1s or 0s than would be expected by chance. With our short time series, this was an inherently difficult assumption to assess (Fig. 2). Most runs that contained more than one observation were for pregnant status, which is to be expected for females of reproductive age, where the probability of pregnancy is much >0.5. A small number of females could have had chronic or acute health issues that led to successive years of non-pregnant status, but it did not appear that observations within a run of non-pregnant observations behaved differently than non-pregnant observations occurring amidst 2 runs of pregnant observations (Fig. 2). Furthermore, we were not overly concerned with potential serial dependence; we maintain that the information in each observation is worth that of an independent measurement regardless of whether the animal was not-pregnant for ≥ 2 successive years.

In our considerations of data independence, we kept our foremost goal to develop a technique for non-invasively predicting the probability of pregnancy from WP at the forefront. Coupling our ultimate goal for the model with the lack of clear evidence for heterogeneity, we considered all observations independent and are confident standard errors accompanying our estimates are not misleadingly small. An important and often overlooked component to our method is the use of uncertainty, and we believe the advantages of incorporating the use of CIs into the process of prediction far outweigh any disadvantages that could arise from a lack of independence in this application.

We evaluated the assumption of linearity between the log odds of pregnancy and the explanatory variable (transformed WP [YC ln(WP)]), by calculating empirical logits for artificially created bins of WP values for data collapsed over females and years. Plots of the empirical logits versus the explanatory variable revealed the linearity assumption was adequately met.

Finally, because our study population was brucellosis-infected, and brucellosis is known to heavily infect the placenta of bison (Cheville et al. 1998, Rhyan et al. 2001; an important producer of progesterone), we were concerned with the possibility that disease status could affect our fecal progestagen measurements. Our question focused on the possibility that fecal progestagen values were not only indicative of pregnancy status, but also of disease status, and that our model could misclassify pregnant animals as non-pregnant due to active brucellosis infections and reduced production of progesterone. Quantitative serology is known to be related to infection in greater Yellowstone area *Brucella*-infected bison (Roffe et al. 1999). To investigate the relationship between infection status and fecal progestagen, we ranked the *Brucella* antibody response of bison as either "high" or "not high" (low or negative antibody response) based on the complement fixation test (Roffe et al. 1999), and compared mean (transformed) WP by serological status, age, and pregnancy status using 128 cases for which we had all 3 variables. We report here comparisons of "high" versus

"not high" antibody response because these are most likely to represent animals with active *Brucella* infections (high) versus those without infections (not high). We found no evidence of an interaction between age and serological status ($P = 0.468$), and we found inconclusive evidence of a difference in mean WP between animals with "high" and "not high" titers after pregnancy status was accounted for (2-sided $P = 0.092$; Fig. 3). We estimated the mean WP for animals with "high" titers to be 0.23 units smaller than for those with "not-high" titers (95% CI = −0.49–0.037). Although we did find evidence of a slight decrease in WP for high-titered animals, the magnitude was not large enough to negatively affect predictions from our model. When simply comparing *Brucella* antibody test-positive versus test-negative individuals, we found no evidence of a difference in mean (transformed) WP.

Procedures were approved by the Animal Care and Use Committee of Montana State University (proposal clearance no. 97-779) and the Biological Resources Division—U.S. Geological Survey, and conformed to the Animal Welfare Act and U.S. Government principles for the use and care of vertebrate animals used in testing, research, and training. Our work was conducted under approved permits by the National Park Service and Wyoming Game and Fish Department.

RESULTS

Reproductive Status

From 1997 to 2005, we assigned pregnancy status using our palpation and calf status criteria and obtained WP values for 155 observations of 42 unique bison; 114 were classified as pregnant and 41 as not pregnant. Years of data for individual females ranged from 1 to 8 ($\bar{x} = 3.7$). Of the 42 unique females, 34 were pregnant in ≥1 year and 25 were not pregnant in ≥1 year. Only 15 females had ≥1 observation for each status. Twelve of the 41 not-pregnant observations came from females below reproductive age (<2 yr), partially explaining the number of females with only non-pregnant observations (Fig. 2). We collected winter fecal samples and determined fecal progestagen levels for all samples. Of the 155 fecal samples, we collected 129 from immobilized animals and 26 from free-ranging individuals.

We obtained blood and conducted pregnancy-specific protein B pregnancy analyses for 129/155 observations for which females were assigned a pregnancy status. Twenty-five of the 129 comparisons between our palpation-calf assigned pregnancy status and pregnancy-specific protein B-derived pregnancy status were in disagreement (Table 1).

Rectal palpation was performed for 123 of the 155 observations that were assigned a pregnancy status (32 non-palpated F were assigned solely based on presence of a calf). Of 41 animals determined not pregnant from palpation, 2 (4.9%) were subsequently documented with calves. The 2 incorrect palpations occurred for observations with the second- and third-longest palpation–birth intervals (186 and 197 days; median of all intervals = 93 days, $n = 81$), which likely rendered fetal detection more difficult. Regardless, based on these data, we estimated a false-negative palpation rate across all palpation–birth intervals as about 5% in our study. In addition to 88 females palpated as pregnant, subsequently observed with a calf, and assigned as pregnant in our analyses, we recorded 24 animals palpated as pregnant that were never observed with a calf. These observations were not used in fitting the model because they did not meet our criteria for assignment as pregnant or not pregnant. Although these cases represented 22.6% (24/106) of observations recorded as pregnant from palpation, the potential for fetal or neonatal loss subsequent to palpations precluded us from estimating a false-positive palpation error rate.

Model Prediction

We investigated the prediction success of multiple models, focusing on including predictors that would be useful for predicting in new, unhandled, unmarked females. For example, age will not be accurately known in these cases; therefore, we had little interest in coming up with a final model for prediction that included it. Mis-prediction rates

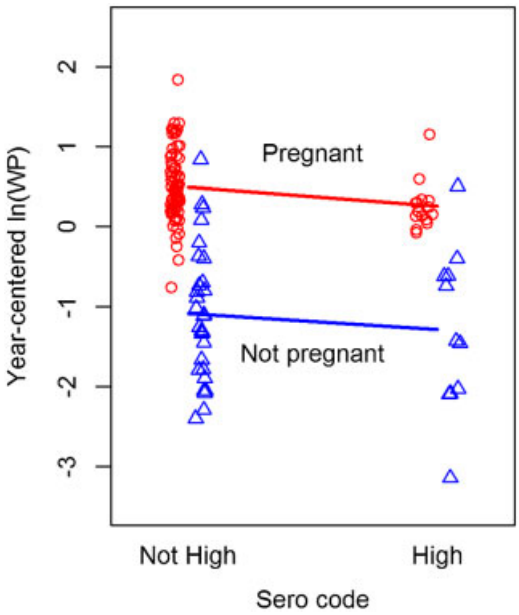

Figure 3. Year-centered, log-transformed values of winter-collected (late gestation) fecal progestagens [ln(WP)] grouped by disease status (Sero Code) and coded by pregnancy status (circle = pregnant, triangle = not pregnant) from the Jackson bison herd, northwest Wyoming, USA, 1997–2005. The lines connect the average for each disease status within pregnancy status.

Table 1. Comparison of pregnancy determination by pregnancy-specific protein B (PSPB) from blood samples and a combination of rectal palpation and annual calf status (assigned status) in Jackson bison, northwest Wyoming, USA, 1997–2005 (shaded cells show disagreement between the 2 methods).

	Assigned status		
PSPB results	**Not pregnant**	**Pregnant**	**Total**
Not pregnant	26	10	36
Pregnant	15	78	93
Total	41	88	129

Table 2. Leave-one-out cross-validation measures of pregnancy status predictive success using winter-collected (late gestation) fecal progestins (WP) in 4 logistic-regression models for Jackson bison, northwest Wyoming, USA, 1997–2005 (YC = yr-centered, WDAT = sample collection date).

Model	% Mis-predicted	Average estimation error	% Mis-prediction CIs not overlapping cut-off	% Correct prediction CIs covering cut-off	Pregnancy *P* cut-off value
WP	14.19	0.283	4.52	27.74	0.5
ln(WP)	12.26	0.223	5.81	13.55	0.5
YC ln(WP)	6.45	0.123	4.52	3.87	0.6
YC ln(WP) + WDAT	7.10	0.117	3.23	5.81	0.5

obtained using cross-validation varied from 6.45% to 14.19% among the 4 logistic-regression models we investigated (Table 2). The models including only year-centered ln(WP) had the lowest mis-prediction rate (6.45%) and the lowest overall percentage of CIs accompanying correct predictions crossing the threshold value (Table 2). Correct predictions were obtained with high confidence using this model and resulted in an overall successful pregnancy prediction rate of 93.5%.

The model incorporating date of fecal-sample collection (WDAT) slightly reduced the average estimation error and the percent of CIs accompanying mis-predictions that did not cross the threshold (Table 2). Fewer wrong predictions were made with this model, but many of these were made with false confidence. On the other hand, the inclusion of WDAT increased the mis-prediction rate from 6.45% to 7.10%. However, the small differences among the results from the models are probably not practically meaningful and either could be used to make predictions. For the following results, we use the model with only YC ln(WP).

The estimated logistic-regression equation using year-centered ln(WP) was

$$\text{Logit}(P) = 1.685 + 3.940 \times (\text{YC ln [WP]})$$

Standard errors for the regression coefficients were 0.338 (intercept) and 0.696 (slope). Performing the inverse logit transformation to obtain the equation for estimating the probability of pregnancy, we obtained

$$\hat{P} = \frac{\exp\{1.69 + 3.94 \times (\text{YC ln [WP]})\}}{1 + \exp\{1.69 + 3.94 \times (\text{YC ln [WP]})\}}$$

The threshold for determining pregnant versus not-pregnant status that resulted in the best predictive success for this model was 0.60, and this was used to make all predictions.

Using the year-centered ln(WP) model, 110 of the 114 observations assigned pregnant status were correctly predicted as pregnant (Table 3). Of the 110, only 3 of the 95% CIs crossed the threshold, which indicated a lack of confidence in the correct prediction. The model correctly predicted 35 of the 41 not-pregnant observations, with 3 CIs crossing the threshold. Of the 10 total mis-predictions, 3 of the associated CIs overlapped the threshold, which indicated an appropriate lack of confidence in the prediction. For those not overlapping the threshold, which indicated false confidence in the predictions, most came very close to the threshold and, thus, in practice would be flagged as uncertain predictions (Fig. 4).

In addition to estimating the probability of pregnancy for a particular female given their fecal progestagen levels, the logistic-regression model allowed interpretation of the relationship between the progestagen level and the odds of pregnancy. As suspected we found convincing evidence

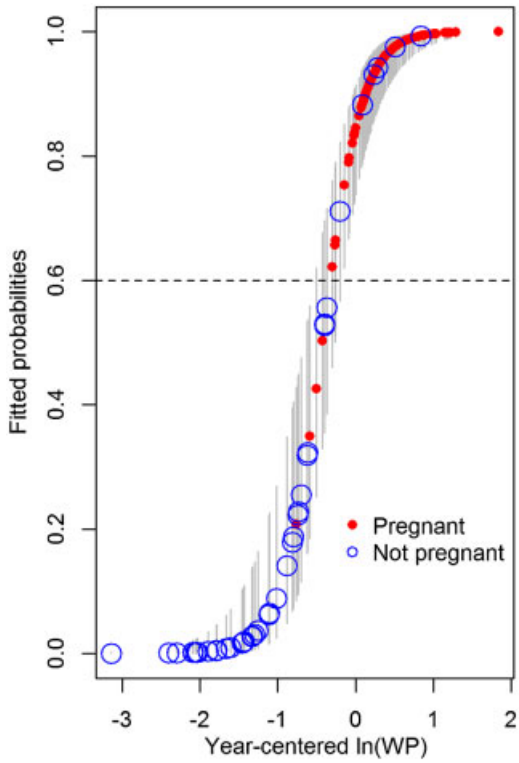

Figure 4. Fitted assigned pregnancy probabilities and 95% confidence intervals for the logistic regression model using year-centered, log-transformed values of winter-collected (late gestation) fecal progestagens (WP) for Jackson bison, northwest Wyoming, USA, 1997–2005 (dashed line represents proposed cut-off for model-predicted pregnancy status).

Table 3. Pregnancy predictions using logistic regression and year-centered, log-transformed values of winter collected (late gestation) fecal progestins (WP) from Jackson bison, northwest Wyoming, USA, 1997–2005.

	Assigned pregnancy status		
Model predictions	Pregnant	Not pregnant	Total
CIs do not overlap cut-off			
Pregnant	107	5	112
Not pregnant	2	32	34
CIs overlap cut-off			
Pregnant	3	1	4
Not pregnant	2	3	5
Total	114	41	155

that year-centered fecal WP level was associated with pregnancy status ($Z_{\text{Wald's}} = 5.66$, $P < 0.001$). A doubling of year-centered WP was associated with an estimated 15.4 ($=2^{3.94}$)-fold increase in the odds of a female being pregnant (95% profile likelihood CI from 6.7 to 45.6 times).

DISCUSSION

Our logistic-regression model provided predictions of mid- to late-gestation bison pregnancy status based on one fecal sample with high accuracy (93.5%). Other studies have reported successful pregnancy-prediction rates based on fecal hormones, of 100% in a small sample of bison (14 pregnant, 4 non-pregnant based on palpation; Kirkpatrick et al. 1992), 85% in captive moose (*Alces alces*; Monfort et al. 1993), approximately 97–100% in elk (Garrott et al. 1998, Stoops et al. 1999), and 93–100% in bighorn sheep (Borjesson et al. 1996, Schoenecker et al. 2004). However, unlike these studies, using logistic regression we derived a measure of confidence for each individual prediction, which allows practitioners to further evaluate estimates for individual animals, and thereby improve prediction interpretations.

The Importance of Independent Pregnancy Measures

Our study underscores the importance of independently validating fecal progestagens with other objective measures of pregnancy when developing pregnancy–fecal hormone relationships. Using calf status alone as an indicator of pregnancy, for example, is not sufficient, because fetal and neonatal losses from predation, disease, or other environmental factors are possible. In our study area, where brucellosis and predators capable of taking bison—wolves (*Canis lupus*) and grizzly bears (*Ursus arctos*)—were common, we documented a 22.6% reduction between bison females palpated as pregnant and those observed with a calf within about a week of birth. Fetal losses due to brucellosis infections in our population could partially explain this reduction, but abortion rates are generally low in chronically infected herds, such as the Jackson and Yellowstone bison herds (Cheville et al. 1998). We also considered the possibility that our palpations could have contributed to fetal losses. To evaluate this, we compared fetal–neonatal loss rates in our bison that were and were not palpated, using the model presented herein to retrospectively determine pregnancy status of animals that were not palpated and not observed with calves. We found that 11/41 (26.8%) pregnant animals fell into this category, which was similar to but slightly higher than the fetal–neonatal loss rate in our palpated sample (22.6%), which suggests that palpation had no effect. Neonatal predation could also have contributed, but our study was not designed to measure this. Nevertheless, our data make it clear that pregnancy estimates based on calf status alone can be prone to underestimating bias.

Transrectal uterine palpation is generally regarded as an accurate indicator of pregnancy status, but its reliability is highly dependent on the experience of the practitioner and the extent of fetal development (Weber et al. 1982). Using experienced veterinarians with mid–late-gestation bison, we documented a 5% false-negative palpation rate, which may have been related to expectations of a larger fetal size at this time in gestation during palpation. The 2 false-negative palpations were in earlier stages of development than May-parturient bison. We were unable to estimate false-positive rates because of confounding factors discussed above. Although the accuracy of palpation is probably sufficient for most studies, we recommend that it be coupled with or substituted by ultrasonography whenever possible to improve our understanding of fecal progestagens as indicators of pregnancy.

Interestingly, based on our pregnancy assignment protocol using both palpation and calf status, we found pregnancy-specific protein B to be an unreliable indicator of pregnancy status. Pregnancy-specific protein B, which Haigh et al. (1991) showed was 93% accurate in wood bison (*Bison bison athabascae*), and which has been used in other studies as an independent indicator of pregnancy status (Garrott et al. 1998, Russell et al. 1998, Joly and Messier 2005), disagreed with 19% (25/129) of our pregnancy assignments based on field measurements, including 10/36 (28%) assigned "not pregnant" by pregnancy-specific protein B and confirmed to have calves later in the season (Table 1). Of these, blood-sample–calf birth-date intervals were 66–78 days for 8 samples, 131 days, and 244 days, which suggests that only the latter case could be suspected of early (gestation) sample-related error. The laboratory that conducted our analyses specified 95% accuracy in bison.

Considerations for Model Refinement

Detailed knowledge of the normal endocrine excretion dynamics of bison was essential for determining the optimal time for sampling (i.e., early- vs. mid- or late-pregnancy). In our study, we used information derived from monthly assessments of urinary and fecal progestagen in bison by Kirkpatrick et al. (1992). However, these data were limited to monthly mean progestagens assessed in 14 females sampled before conception through the first 4.5 months of gestation (Sep, Nov, and Jan). Sampling strategies for pregnancy detection would likely benefit from more comprehensive longitudinal assessments of fecal progestagens excretion in pregnant and non-pregnant females sampled before and throughout the entire period of gestation. Aligning sampling effort with the gestational stage when females excrete maximal fecal progestagens would increase the likelihood of discriminating pregnant from non-pregnant or cycling females (Monfort et al. 1993). Furthermore, more intensive sampling, perhaps in a captive herd, would help to elucidate the extent of among-individual variation, including age-related effects on fecal hormone production. Nevertheless, our model produced a useful predictor of pregnancy in bison with a single mid–late-gestation fecal sample.

Fecal progestagens vary in non-pregnant, reproductively active female bison, reaching a peak during the luteal phase when the corpus luteum is producing progesterone. Though bison are seasonally polyestrus in the late summer/early fall, we occasionally observed young (<3 months old) calves, and males tending apparently cycling females, during our winter captures. We made no attempt to ascertain the reproductive

status of non-pregnant bison (anestrus vs. actively cycling), but this suggests the possibility of non-pregnant bison with high WP in our sample. Some of the bison designated not pregnant by our field criteria but model-predicted to be pregnant because of high WP levels may have been reproductively active. Had we been able to control for this variable, our model may have been improved.

MANAGEMENT IMPLICATIONS

Knowledge of pregnancy rates is often lacking in demographic models used to manage ungulate populations, but their inclusion would greatly increase the informative power, problem-solving attributes, and overall utility of such models. While our study results are directly applicable to bison, the approach will facilitate the work of wildlife biologists, researchers, reserve managers, and others by providing an effective and efficient method for estimating pregnancy—and assessing the associated uncertainty in the estimates—in free-ranging ungulates at scales ranging from individual animals to populations. The approach is useful to practitioners because fecal samples are easily collected and preserved, laboratory procedures are well-documented, and logistic regression is readily available in statistical computer software. Furthermore, samples can be obtained non-invasively, without the need to handle animals and incur drawbacks associated with immobilization such as cost, human and animal safety, potential data bias, and lack of social acceptability. The benefits of the latter should not be underestimated in contemporary wildlife management or research settings, given the increasing concern among the general public and special interests for manipulation of wild animals (see Peterson et al. 2003 and Farnsworth and Rosovsky 1993).

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