Conservation Endocrinology: a Noninvasive Tool to Understand Relationships between Carnivore Colonization and Ecological Carrying Capacity

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Abstract: *Reproductive technology, especially the diagnosis of pregnancy by radioimmunoassay of fecal steroid metabolites, is an important component of captive propagation, but its role in our understanding of ecological interactions and in situ biological restoration has been more limited. Where large herbivores have been "released" from predation by the extirpation of carnivores, controversy often exists about possible detrimental effects at the ecosystem level. A related concern is that the reestablishment of large carnivores may decrease the availability of prey populations for human subsistence. We suggest that pregnancy assays can be a valuable tool to help distinguish between the roles of predation versus food-imposed limitations on population size and their effects on juvenile recruitment in wild species. We explored this issue through analyses of fecal progestagen concentration (FPC) levels to document pregnancy in moose (*Alces alces*) in the southern Greater Yellowstone Ecosystem, a site where wolves (*Canis lupus*) and grizzly bears (*Ursus arctos*) are recolonizing former habitats after an absence of more than 60 years. Pregnancy was clearly discernible (mean FPC for pregnant and nonpregnant females, respectively: 10.60 vs. 2.57 $\mu g/g$;* $p < 0.0001$*). Among the potential confounding variables that need to be considered if FPC is applied to ecological and demographic questions are whether baseline values are affected by handling, whether neonate survival has been assessed, and whether sampling efforts are directed at both pregnant and nonpregnant animals. With these issues accounted for, the local moose population experienced juvenile survival rates among the highest in North America. Pregnancy rates, however, dropped from 90% in 1966 to about 75% today, rendering them in the lowest fifteenth percentile among moose populations in North America. Our findings suggest that a relatively low frequency of juvenile moose is not the likely result of predation, and they illustrate how endocrinology can be applied to issues involving reproductive events within an ecological context. They also affirm that noninvasive and generally inexpensive endocrinological procedures will be applicable to understanding interactions between recolonizing predators and prey, an issue that will continue to arise because of global restoration efforts, and to the study of rare ungulates in remote systems where data on reproductive events are difficult to obtain.*

Endocrinología en Conservación: una Herramienta no Invasiva para Entender las Relaciones entre Colonizacíon de Carnívoros y la Capacidad de Carga Ecológica

Resumen: *La tecnología reproductiva, especialmente el diagnóstico de embarazo por radioinmunoensayo de metabolitos fecales es un componente importante de la progación en cautiverio, pero su papel en el entendimiento de interacciones ecológicas y en restauraciones biológicas* in situ *ha estado mas limitado. En sitios donde se he "liberado" a herbívoros grandes mediante la extirpación de carnívoros, se presentan frecuente-*

Paper submitted November 5, 1998; revised manuscript accepted April 21, 1999.

mente controversias sobre los posibles efectos negativos a nivel de econsistema. Una preocupación relacionada es que el re-establecimineto de carnívoros grandes podría disminuir la viabilidad de poblaciones de presas para subsistencia humana. Nosotros sugerimos que los análisis de preñez pueden ser una herramienta valiosa para ayudar a distinguir entre los papeles de depredación contra limitaciones de alimento impuestas en el tamaño poblacional y sus efectos en el reclutamiento de juveniles en poblaciones silvestres. Exploramos este tema mediante el análisis de niveles de concentración de progestágenos fecales (FPC) para documentar embarazos en alces (*Alces alces*) en el ecosistema sureño del gran Yellowstone, un sitio donde los lobos (*Canis lupus*) y osos grizzly (*Ursus arctos*) estan recolonizando hábitats anteriormente usados, después de una ausencia de mas de 60 años. El embarazo fué claramente discernible (FCP promedio pra hembras preñadas y no preñadas respectivamente: 10.6 vs 2.57 mg/g; $p < 0.001$). Entre las variables potencialmente confundentes que necesitan ser consideradas si el FCP es aplicado en situaciones ecológicas y demográficas estan el saber si los valores base son afectados por el manejo, evaluar la supervivencia de neonatos y dirijir los esfuerzos de muestreo tanto a animales preñados como no preñados. Con estos aspectos tomados en consideración, la población local de alces ha experimentado tasas de supervivencia de juveniles entre las mas altas de Norteamérica. Sin embargo, las tasas de embarazo disminuyeron de un 90% en 1966 a cerca de 75% actualmente, colocándolas en el quinceavo percentil mas bajo entre las poblaciones de alces de Norteamérica. Nuestros resultados sugieren que la relativamente baja frecuencia de alces juveniles no es una posible causa de la depredación, e ilustran como la endocrinología puede ser aplicada en asuntos relacionados con eventos reproductivos dentro de un contexto ecológico. Esto también afirma que los procedimientos endocrinológicos no invasivos y generalmente baratos pueden ser aplicados para: Entender las interacciones entre depredadores recolonizando y sus presas, un asunto que continuará sugiendo debido a los esfuerzos globales de restauración, y el estudio de ungulados raros en sistemas remotos donde los datos de eventos reproductivos son dificiles de obtener.

Introduction

Captive propagation typically requires a comprehensive understanding of metabolic aspects of reproduction (Kleiman 1980; Hutchins et al. 1996). Among the techniques used to assess pregnancy are assays of blood sera, including pregnancy-specific B protein and chorionic gonadotropin (Neill & Knobil 1972; Sasser et al. 1986); ultrasound and laparoscopy (Adams et al. 1991); urinary (Hodges et al. 1979; Monfort et al. 1987) and fecal (Desaulniers et al. 1989) steroid assays; and palpation of the reproductive tract. Although such methods are used routinely in zoos, they are only now being applied to wild populations (Lasley & Kirkpatrick 1991), partly because of logistics, expense, and concerns about invasive handling procedures (Bekoff & Jamieson 1996). Nevertheless, noninvasive monitoring of pregnancy has been used successfully for free-roaming baboons (*Papio cynocephalus*; Wasser et al. 1991), bison (*Bison bison*; Kirkpatrick et al. 1993), dwarf mongooses (*Helogale parvula*; Creel et al. 1991), African wild dogs (*Lycaon pictus*; Creel et al. 1997), and elk (*Cervus elaphus*; Garrott et al. 1998). Such monitoring can expand knowledge about population and species management into the realm of ecosystems.

In terrestrial environments, a first tier of extirpations involves large carnivores, and these losses often result in increased prey densities (Terborgh 1988; Wright et al. 1994). Among protected areas that vary from the Swiss Alps and African savannas to North American prairies and mountains, debate exists about the possible effects of predation on prey and whether species freed from predation influence biodiversity (Garrott et al. 1993; McShea et al. 1997). In Grand Teton, Rocky Mountain, and Yellowstone national parks, for instance, wolves (*Canis lupus*) and grizzly bears (*Ursus arctos*) were reduced or eliminated, and prey species (including elk, moose [*Alces alces*], and bison) are now at their highest recorded levels and may be affecting plant communities (Wagner et al. 1995). Although the extent to which the loss from or restoration of predators to a system creates a new "equilibrium," or whether an equilibrium exists at all, remains controversial (Despain et al. 1986; Brussard 1992; Kay 1994; Coughenour & Singer 1996), it is clear that the reintroduction or natural recolonization of carnivores creates experimental opportunities to investigate processes involving ecosystem dynamics and health.

In the Greater Yellowstone Ecosystem, where wolves and grizzly bears are predators of ungulates, scientific opinion is divided about the extent to which predation, nutrition, and other factors may limit large herbivores (Table 1). And because both grizzly bears and wolves are recolonizing areas outside national parks, there are also practical reasons for wishing to understand the ecological consequences associated with the reestablishment of large carnivores in areas where they have been previously extirpated. These include (1) enhanced susceptibility of naive prey to predation (Berger 1998); (2) reduction of potentially harvestable species to the hunting public because carnivores kill big game (Orians et al.

1997); (3) increased ecosystem effects because tourism increases where carnivores are observable (Bath 1991); and (4) compromised economic interests in the private sector because of predation on livestock.

Our aim is to illustrate two points: (1) the advantages and practical limits of using fecal steroid metabolites to facilitate an understanding of pregnancy in a wild ungulate and (2) through example, how such results may contribute knowledge about the relative role of predation and food limitation in a system as recolonization and geographical expansion of large carnivores occurs.

The use of modern, noninvasive endocrine technology has implications broader than those for the Yellowstone region alone. For instance, the handling of wild animals is discouraged in many U.S. national parks. And, in inaccessible areas, such as the Annamite region of Laos and Viet Nam where new ungulate species have recently been discovered (Schaller & Rabinowitz 1995; Schaller & Vrba 1996), monitoring through fecal collection may be one of the few logistically effective and noninvasive ways in which pregnancy can be diagnosed. Finally, the restoration of large carnivores has been underway in North America for at least four decades, and it continues in Europe and Africa (Reading & Clark 1996). Inevitably, questions will continue to arise about both the dynamics of predator-prey interactions and ecosystem-level effects (Table 1). The use of fecal and urinary by-products holds promise for enhancing knowledge about the extent to which food, predation, and other factors affect reproduction and population levels.

Our efforts focused on moose, a species that is greatly affected by both grizzly bears and wolves (Orians et al. 1997), that occurs at low densities in Yellowstone National Park, where it is unclear whether predation or nutritional limitation restricts population size (Tyers & Irby 1995; Mattson 1997), and whose status in northwestern

Table 1. Summary of selected examples of tests to identify primary factors that may influence population levels, including fecal progestagen concentration (FPC) of large herbivores in the Greater Yellowstone Ecosystem and on other continents.

Factor	*Species*	*Location*	*Prediction*	*Evidence or comment**
Greater Yellowstone Ecosystem				
disease	elk	Jackson Hole	disease limits population increase	not supported because the diseased population increases (1–3)
	bison	Jackson Hole	disease limits population increase	as above (4)
predation	moose	Jackson Hole	pregnancy rates should be relatively high	not supported because pregnancy rates are low (this study, using FPC)
food	elk	Yellowstone Park	population stabilizes at an "equilibrium" level (5)	mixed; winter regulation of populations size (2, 5–7)
Other ecosystems				
disease	wildebeest	Etosha, Namibia	in absence of anthrax, populations increase (8)	mixed; as disease decreased, population did not increase (8–10)
	wildebeest	Serengeti, Tanzania	in absence of rinderpest, populations increase (11)	supported (12)
predation	topi	Serengeti, Tanzania	with lion removal, juveniles increase (13)	supported by contrasts of protected and unprotected populations (13)
	impala	Serengeti, Tanzania	with lion removals, juveniles increase (13)	supported by contrasts of protected and unprotected populations (13)
	moose	Alaska, & Ontario, Canada	with carnivore removal, juveniles increase (14)	supported by contrasts of exploited and protected populations (14)
food	zebra	Etosha, Namibia	regulation manifested by negative feedback on birth or survival rates (13, 15)	not supported; recruitment unchanged during drought and non-drought (10)
	springbok	Etosha, Namibia	regulation manifested by negative feedback on birth or survival rates (13, 15)	not supported because of high pregnancy rates (10)
	white-tailed deer	George Reserve, U.S.A.	regulation manifested by negative feedback on birth or survival rates (13, 15)	supported by reduced birth rate (16)
	red deer	Rhum, Scotland, U.K.	regulation manifested by negative feedback on birth or survival rates (13, 15)	supported by increased mortality (17)

**References: 1, Boyce (1989); 2, Houston (1982); 3, Smith & Anderson (1998); 4, Berger & Cain (1999); 5, Boyce (1991); 6, Singer et al. (1997); 7, Coughenour & Singer (1996); 8, Berry (1981); 9, Lindeque (1991); 10, Gasaway et al. (1996); 11, Sinclair (1977); 12, Sinclair (1979); 13, Sinclair (1995); 14, Messier (1994); 15, Sinclair, (1989); 16, McCullough (1979); 17, Clutton-Brock & Albon (1989).*

Wyoming is likely to be affected by wolf recolonization both within and beyond Yellowstone and Grand Teton national parks (Wyoming Game and Fish 1997). Because juvenile recruitment in moose is suspected of being low at sites of sympatry with grizzly bears (J. Bohne, personal communication), it is possible that neonates are being lost to grizzly bears, as has been documented in Alaska and elsewhere (Gasaway et al. 1992; Orians et al. 1997). Concern exists that wolf recolonization may diminish prey populations now harvested by humans (Wyoming Game and Fish 1997). Although increased predator densities can easily be imagined to exacerbate ungulate mortality, other factors may also be involved. The alternate hypothesis, that inadequate food supply limits fecundity and hence neonate production, is in need of testing (Table 1).

The food limitation hypothesis is amenable to empirical examination because the noninvasive monitoring of fecal steroid hormones permits an unbiased measure of pregnancy. Our working assumption, that females in populations at or above a minimum subsistence level of food are in poor(er) physiological condition and characterized by reduced pregnancy rates compared to females in populations where predation regulates population size, has been confirmed in many large herbivores (Clutton-Brock & Albon 1989; Sinclair 1989), including moose (Franzmann & Schwartz 1985; Gasaway et al. 1992).

Methods

During the winters of 1996–1998, we anesthetized 25 adult female moose in Grand Teton National Park and on the National Elk Refuge, a part of the southern Greater Yellowstone Ecosystem in northwestern Wyoming. We used a carfentanil/xylazine drug combination delivered in a dart for immobilization. Pregnancy was initially diagnosed by transrectal palpation (TR) of the reproductive tract. The timing of our sampling (midwinter) permitted direct detection of the fetus in pregnant animals. Subsequently, pregnancy was also assessed by association with fecal progestagen concentration (FPC). Thirty-seven additional fecal samples were gathered from females (24 adults, 7 calves, 6 yearlings). All samples were obtained from 14 December until 30 April and enabled either initial assessment ($n = 37$) or reassessment ($n = 25$) of pregnancy status. All females were outfitted with radio collars (Advanced Telemetry System, Isanti, Minnesota), with which we were able to track their locations from winter until late July or August of each year. Females were palpated only at the time of radio-collaring, not in subsequent years, and these females constituted the sample of 37 in which FPC was determined based on collection of feces in the field during later years. Detailed follow-up and daily observations of as many females as possible yielded the information on whether these radio-collared females gave birth. Calf ages were known by birth date or estimated based on steadiness while standing, retention of the umbilicus, and ability to run, or on the number of days between sightings of mothers before and after birth.

Fecal samples (approximately 5 gm) were stored frozen in plastic bags. Processing, extraction, and radioimmunoassay (RIA) procedures (Wasser et al. 1994) have been previously validated for captive moose (Monfort et al. 1993; Schwartz et al. 1995). Hormone concentrations are expressed as mass units of hormone excreted per gram of dry feces.

We classified anesthetized females as either "known" pregnant or not pregnant based on palpation. For animals not subsequently handled, pregnancy status was provisionally listed as either "suspected" pregnant or not pregnant based on fecal progestagen levels relative to known samples (Fig. 1). We recognized and attempted to minimize several potential sources of error. First, luteal levels may be indistinguishable at early stages of pregnancy. Thus, our fecal sampling procedures did not begin until mid-December, several months after the early fall rut commenced and during a period when pregnancy would have been further along. Second, nontarget fecal samples could have been gathered erroneously. That is, the specimens may not have resulted from defecation by the desired radio-collared individual but by an adjacent female. This possible source of error was reduced in the present analysis by restricting

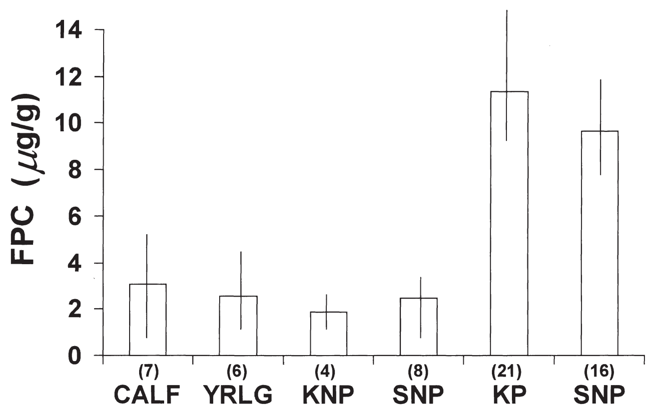

Figure 1. Relationships between mean fecal progestagen concentration (FPC) and 95% confidence levels (vertical bars) for six categories of live female moose from Grand Teton National Park and the National Elk Refuge, Wyoming. (KNP, known not pregnant; SNP, suspected not pregnant; KP, known pregnant; SP, suspected pregnant; CALF, calf; and YRLG, yearling.) Suspected pregnant and nonpregnant animals were not anesthetized for independent evaluation, and suspected status is based on differences in FPC between animals that were handled.

acceptable samples only to those based on direct observation or collection in snow when no other animals were present. Third, the pregnancy assessment by palpation might have been wrong, resulting in either a false positive or a false negative. Of 21 immobilized females initially designated as pregnant, 85% (17 of 20; 1 subsequently died of starvation) had calves; none of 4 that were palpated and assumed not to be pregnant had calves. Thus, in a worst-case scenario, out of 24 diagnoses there would have been 3 misdiagnoses (13%), an outcome that does not take into account potential in utero fetal loss or mortality immediately after birth. By determining the FPC of animals judged to be pregnant through palpation but that were not observed with neonates, it is possible to assess the probability of an incorrect pregnancy classification.

Results and Discussion

Fecal Progestagens and Pregnancy

Females classified as pregnant prepartum by rectal palpation (known pregnant, KP) had FPC levels that exceeded known nonpregnant (KNP) females by more than five times (Fig. 1; $p < 0.001$). For nonanesthetized females, pregnancy status was assigned as either suspected pregnant (SP) or suspected nonpregnant (SNP) based on FPC (Fig. 1). Using these criteria, no within-group (i.e., KP vs. SP and KNP vs. SNP) differences were detected, but striking overall variation in FPC existed between pregnant and nonpregnant females (one-way analysis of variance; $F_{5,61} = 11.86$; $p < 0.0001$). Little within-category variation in FPC existed for any of the four nonpregnant categories—calves, yearlings, and females either known or suspected to be pregnant ($p = 0.943$)—or two pregnant ones (KP or SP; $p = 0.423$; post hoc multiple comparisons with Student-Newman-Keuls test).

These results suggest that study of pregnancy, as previously described in captive moose (Monfort et al. 1993; Schwartz et al. 1995), can now be extended to wild conspecifics. If, however, the use of FPC is to be useful for assessment of pregnancy in wild populations, the effects of several potentially confounding variables must be accounted for, including (1) the intensity of sampling; (2) the accuracy of pregnancy assessment; and (3) relationships between the handling of animals and development of baseline values.

First, if our efforts to detect newborn calves differed between categories of females and resulted in a failure to detect newborn calves (because they had died), then females could have been assigned to an incorrect pregnancy category. Hence, it is critical to know whether calf production varied by FPC. To evaluate this prospect, we contrasted day of first observation of newborns between two categories of females, those of suspected and of known pregnancy status; there were no differences (means, respectively, were 7.5 ± [SEM] 2.4 days [$n = 13$] and 6.2 ± 1.6 [$n = 11$]; $t = 0.50$; $p = 0.63$; median for both categories = 4). Similarly, there was no difference ($p = 0.5543$, Fisher's exact probability test) in offspring production between females known to be pregnant (e.g., handled; 85% of 20) and those suspected of being pregnant (unhandled; 81% of 16). Although the possibility of a Type II error exists because a 4% difference in actual calf production might be statistically significant (at the $p = 0.05$ level), to demonstrate this would require an unrealistic sample of 1178 adult females. We believe that the magnitude of variation in calf production is small.

Second, although we failed to detect differences in calf production between females classified as pregnant by means of both palpation (85%) and FPC (81%), we might have incorrectly deemed handled females as not pregnant when indeed they were. This possibility can be assessed by assuming high postnatal survival and checking whether females diagnosed as nonpregnant produced calves. None of 12 adults (4 handled, 8 unhandled), all with low FPC (Fig. 1), had offspring. Although these animals might have produced calves that died soon after birth, this scenario seems unlikely because calf survival in our study area approached 95% (Berger 1998).

It is also possible to check the extent to which our presumption of accurate pregnancy diagnoses compares to that in other populations by contrasting prenatal losses in our study population with those in others. That is, if our failure to detect births resulted in unusually high frequencies of no births of putatively pregnant animals, then it is likely that our assessments of pregnancy were in error. Alternatively, if interpopulation differences are not evident, then it is unlikely that sampling error explains our results for pregnancy.

We contrasted the number of undetectable births in the Teton population (6 of 36 possible) with that from the Talkeetna Mountains in Alaska, where pregnancy was assessed by blood sera or ultrasonography (Stephenson et al. 1995; Testa 1998; Testa & Adams 1998). Between 1995 and 1997, 86% ($n = 176$) of pregnant Alaskan females produced young, whereas 83% of the Jackson Hole females did, variation that does not approach significance ($z = 0.029$; $p < 0.95$). Our data fell within the range of apparent prenatal losses in Alaska.

We performed an additional check to assess the degree to which FPC is affected by sampling date. If date and FPC are correlated, then date (rather than FPC) might account for differences in our assessment of pregnancy via fecal steroids. Nevertheless, only for females that we deemed pregnant and detected subsequent calves was there a strong relationship between date and FPC, with date explaining 37% of the variance (Fig 2). For nonpregnant animals, a weak, inverse nonsignificant

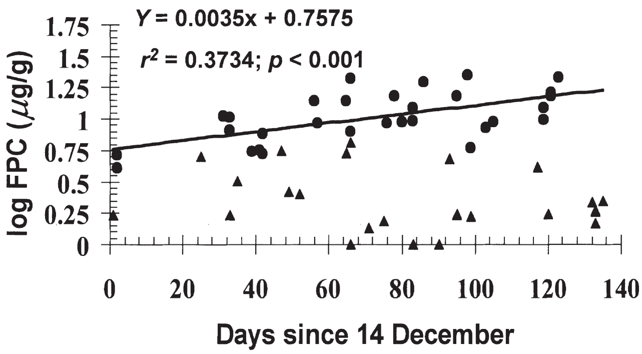

Figure 2. Relationship between sampling date and log fecal progestagen concentration (FPC) in adult female moose (solid circles) known to have given birth. The explanatory power of date of collection on FPC is reduced ($r^2 = 0.25$; $y = 0.0777x + 4.394$; $p < 0.001$) when females ($n = 6$; not shown) suspected of being pregnant but that were not subsequently observed with neonates are included in the statistical analyses. Solid triangles are nonpregnant animals.

relationship was detectable ($y = -0.0015x + 0.4699$; $p > 0.20$), but date of sampling explained little of the variation in FPC ($r^2 = 0.054$; Fig. 2).

For pregnant females, FPC might also vary not only in relation to sampling date but by year. To examine this possibility we used partial correlation to remove the effects of sampling date and then contrasted FPC among females with calves in different years. Year of collection had a greater influence on FPC ($r = 0.524$) than did sampling day ($r = 0.348$), but both factors exerted strong independent effects ($p < 0.001$). In 1998, mean FPC for pregnant females was higher ($\bar{x} = 14.91 \pm 1.43$) than in prior years (1996: $\bar{x} = 8.19 \pm .50$; 1997: $\bar{x} = 7.79 \pm 0.77$; $F_{2,25} = 8.48$; $p < 0.011$); values for 1996 and 1997 did not differ from each other ($p = 0.792$). Overall FPC (1996–1998) of pregnant females exceeded that for nonpregnant females (Fig. 1). Annual variation was not detectable among nonpregnant females ($F_{2,21} = 0.51$; $p = 0.607$).

Third, perhaps our one-time handling of individual animals decreased annual reproductive output or altered FPC. If this concern were valid, then neonate production should vary between handled and unhandled females, but it did not (85% vs. 81%; see above). Finally, because methodologies (for capture, immobilization, collection, and analysis) did not differ among years, our baseline values are likely to reflect biological variation in relationship to pregnancy.

Pregnancy and Juvenile Survival

Our analyses reflect pregnancy rates for a moose population that expanded in a wolf- and (essentially) grizzly bear-free environment for about 60 years. Current pregnancy rates (75.5%; $n = 49$; 1996–1998) are lower than the 90.2% ($n = 41$) reported for the same population between 1963 and 1966 by Houston (1968). The change we found was not quite significant (G Test for Independence; $G_{adj} = 3.36$; $0.05 < p < 0.10$), but statistical power was low due to the small sample size. Nevertheless, the direction of the decline suggests a diminution in rates of pregnancy. Whether density dependence operating through the effects on population size per se has contributed is uncertain.

Although grizzly bears have enlarged their range from Yellowstone National Park and have colonized parts of the Jackson Hole region and adjacent areas since about 1990 (S. L. Cain, unpublished data), predation on adult moose by grizzlies was not confirmed until 1996. Wolves arrived only in late 1998 (J.B., unpublished data). Calf survival currently may be affected by these predators, by mesocarnivores in the recent past including black bears (*U. americana*) or coyotes (*C. latrans*), or by other factors. We evaluated a component of this proposition by examining whether the loss of grizzly bears and wolves affected calf survival. We contrasted 10 populations at sites that varied in the presence or absence of these large carnivores (Fig. 3). Three sites lacked wolves and grizzly bears, and our study area had no wolves and colonization by grizzly bears was occurring. Mean calf survival for radio-collared females in the southern Greater

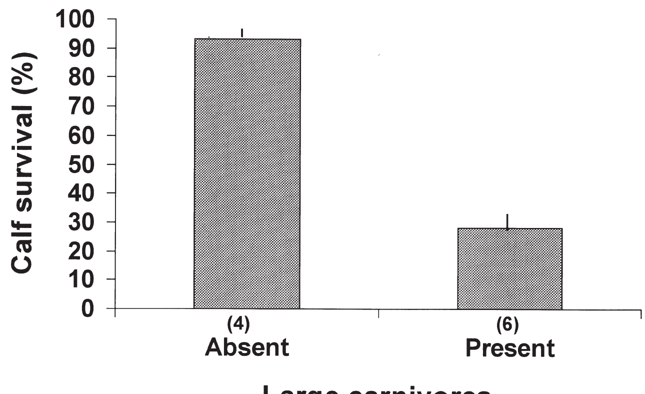

Figure 3. Comparison of mean and standard error of the mean (vertical bars) survival rates of moose calves from Norwegian, Canadian, and American sites with and without grizzly bears and wolves. Carnivores absent, 4 sites: Bridger-Teton National Forest and Grand Teton regions of Wyoming (J.B., unpublished data) and Marks and Robertsfors regions of Norway (Swenson et al. 1999). Carnivores present, 6 sites: Talkeetna (Testa 1998), Denali (Bowyer et al. 1998), Susitna (Ballard et al. 1991), Tok (Gasaway et al. 1992) regions of Alaska; Yukon site in Canada (Larsen et al. 1989); and Orsa in Norway (Swenson et al. 1999).

Yellowstone Ecosystem (96%) and three other study sites (93.3 ± 1.9) was significantly greater than in the six sites with grizzly bears and wolves (28.3 ± 6.9%; t = 7.45; $p < 0.0001$). In the absence of grizzly bears and wolves, moose calf survival is improved.

Ecological Questions and Endocrine Applications

Although concern rightfully exists about the extent to which it is necessary for humans to risk killing animals to study them (Bekoff & Jamieson 1996), noninvasive methods are available and realistically can be used to study pregnancy in wild populations. For FPC to be useful, however, multiple issues need to be addressed. We offer a guide to these concerns by outlining in question format decisions that inevitably will help determine whether one's data can be used to estimate pregnancy in wild populations (Table 2). For instance, among potentially confounding variables that need to be not only considered but also resolved are whether pregnancy can be assessed independently from FPC, whether baseline FPC values are affected by handling, if births actually occur, and whether sampling efforts are directed at both pregnant and nonpregnant animals so that some estimate of error is possible (Table 2). Wherever new technologies are applied in science, care is obviously required to ensure adequate sampling, rigorous use of hypothetical-deductive logic, and appropriate consideration of controls.

Even when such precautions have been exercised, logistical and methodological issues remain. First, some degree of initial handling of animals will always be required because pregnancy must be assessed independently. Also, if individuals are not subsequently identified and feces are collected in the field without a rigorous sampling procedure, the degree to which the data reflect just a few individuals or a cross section of the population will be unknown. Second, FPC profiles not only will vary interspecifically but there is likely to be variation associated with populations, perhaps due to ecological and weather-related conditions. Third, diet (captive vs. wild) may also contribute, as might inherent differences among laboratories. Fourth, while the fecal samples we gathered were fresh because we needed to confirm the association between known individuals and their feces, the effects of time and weather on endocrine profiles of feces remaining in the field for differing time frames remain unclear. Finally, fecal steroid analyses are not as reliable during early stages of pregnancy, and progestins may remain elevated for a period after embryonic death and resorption, topics that require additional study if the techniques we report are to be applied broadly.

Despite these caveats, FPC is useful in assessing pregnancy in wild animals (Figs. 1 & 2) and entails implications beyond reproductive assessment. For instance, if herbivore populations are food-limited (as often is suspected when large carnivores have been eliminated), if hunting by humans does not occur, and if adult mortality is low, then pregnancy rates should be reduced relative to those in populations with heavy predation (Table 1). On the other hand, for populations in which food is more abundant, pregnancy rates should be higher, whether or not predation or other factors restrict population size. Depending upon the species, population, and local environment, empirical evidence is available to support multiple processes (Table 1).

The public and government agencies of the Greater Yellowstone Ecosystem have focused on possible effects on prey population of the geographical expansion of grizzly bears and wolves (Boyce & Keiter 1991; Brussard

Table 2. Summary of questions and comments to be addressed in application of fecal progestagen concentration (FPC) to pregnancy assessment in wild populations

Question	*Comment*
(1) Has pregnancy independently been assessed using methods other than FPC?	required for FPC to be most useful
(2) Has FPC been verified in relation to pregnancy in a control setting?	not absolutely necessary if (1) is done, but FPC and pregnancy must be associated
(3) Is baseline FPC affected by handling?	extent of change will affect utility of FPC application
(4) Has neonate survival been assessed?	required for FPC to be most useful
(5) Are rates of birth similar between handled and non-handled animals?	if so, FPC may be useful; if not, differences may be due to handling and thus render use of FPC less efficacious
(6) Does effort to detect neonates differ by female status?	if so, then it may not be possible to know if neonates died or were not born
(7) Have dates of sampling and possible individual, seasonal, and annual effects on FPC been controlled?	required for FPC to be useful
(8) Are data on putative prenatal losses comparable to other populations?	if so, there is comfort; if not, causes of variation may be unclear*

**Even with a high concordance of data on prenatal losses between sites, many other variables could be involved.*

1992; Phillips & Smith 1996). In areas beyond the protected boundaries of Grand Teton and Yellowstone national parks, human hunters and predators may compete for access to the same ungulate food sources, as has been reported in Montana (Boyd et al. 1994), Alaska (Gasaway et al. 1992; Orians et al. 1997), and the Neotropics (Jorgenson & Redford 1993). Although prey populations will inevitably respond at some level to recolonizing large carnivores, it remains unclear whether concern about the survival of juvenile moose is warranted, particularly in the face of uncertainty about whether females are pregnant at all.

Our data on FPC make it clear that current pregnancy rates (~75%) in moose in the southern part of this ecosystem are low, in the bottom fifteenth percentile of North American populations (Fig. 4), a drop from the 90% that occurred 30 years ago (Houston 1968). Although it is unclear how weather, food, and other factors contribute to the present relatively low pregnancy

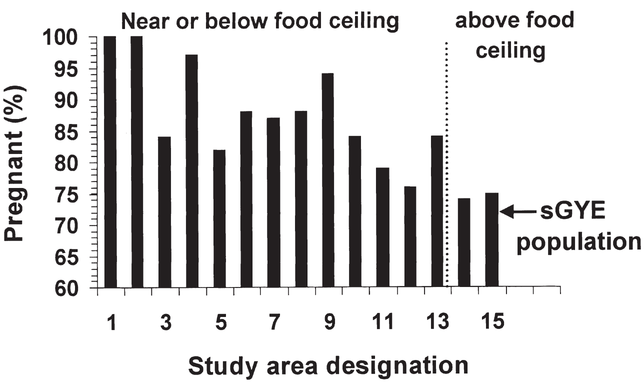

Figure 4. Relationship between percentage of pregnant moose and their food ceiling in 14 populations from Alaska and Canada (modified from Gasaway et al. 1992) and moose from the present study. Food ceiling is a relative measure in relationship to the population growth rate as designated by Gasaway et. al. (1992, 1996). Dotted line separates populations near or below the food capacity from those suspected of being in excess of it. Study area designation (sample size): 1, east central Alaska (28); 2, Innoko River, Alaska (17); 3, Alaska Peninsula (57); 4, Pukaskwa, Ontario (37); 5, Elk Island, Alberta (28); 6, south central Alaska (64); 7, east Newfoundland (29); 8, central Alaska (35); 9, south central Alaska (87); 10, southern Yukon (58); 11, New Brunswick (52); 12, British Columbia (80); 13, Elk Island, Alberta (216); 14, Sandy-M-Town, Newfoundland (87); 15, southern Greater Yellowstone Ecosystem (sGYE), Wyoming (49). For two populations (Elk Island and South Central Alaska), pregnancy rates available for each, but during two time periods that differed by at least a decade and these are shown separately.

rates, populations that are near or below their ecological carrying capacity due to predation by either natural carnivores or humans have greater pregnancy rates than populations exceeding their food base (Caughley 1976). Of the 15 wild populations in Fig. 4, only the one in Newfoundland had been described as above the food ceiling (Gasaway et al. 1992). Although we have not directly assessed food availability in the southern Greater Yellowstone Ecosystem, the decline in pregnancy rates, apparent lack of significant population growth, and general absence of predation on moose is not inconsistent with the possibility of food limitation.

We have not provided an explicit test of factors that affect moose populations. Our goal has been to show how the assessment of pregnancy via analyses of fecal metabolites, especially when coupled with comparative data, can lend itself to a broader understanding of populations, whether they be prey, predators, or occupants of other trophic positions. If food limitation occurs for some ungulates in the Greater Yellowstone Ecosystem as the size of large carnivore populations increases, changes in fecundity should also occur. Although it may take years to detect any putative broad-scale effects on reproduction, this and other predictions about reproductive events are now testable with noninvasive endocrine technology. With large carnivores being re-introduced to or recolonizing this and other ecosystems where they once existed, noninvasive monitoring techniques based on excretory byproducts should become a more important tool for evaluating not only population dynamics but also ecological relationships.

Acknowledgments

We thank D. Brimeyer, S. Cain, C. Cunningham, K. Coffin, G. Roffe, and B. Smith for help in the field. Support was provided by the National Park Service, the Eppley Foundation, the University of Nevada, Molly Knudtsen, the National Science Foundation, and the Wildlife Conservation Society. For comments on this manuscript, we thank S. Cain, C. Cunningham, D. Kleiman, E. Main, D. Murray, K. White, and two anonymous reviewers. This study was facilitated by the cooperation of the Wyoming Department of Game and Fish (J. Bohne and B. Holtz), the U. S. National Park Service (S. Cain, R. Schiller), and the U. S. Fish and Wildlife Service (B. Reiswig).

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