RISK EFFECTS IN ELK (*Cervus elaphus*): BEHAVIORAL AND NUTRITIONAL RESPONSES TO WOLVES AND ENVIRONMENTAL CONDITIONS

by

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ABSTRACT

Until recently, predators have been though to regulate prey primarily through direct predation, in ecosystems where top down effects have been shown to be important. However, experiments and recent empirical observations show that the costs of antipredator responses in individuals that successfully avoid predation can exert equal or larger driving forces on population dynamics than the numerical effect of direct predation. Such a mechanism has not been explored in a large terrestrial vertebrate. I explored the antipredator responses of elk (*Cervus elaphus*) to wolves (*Canis lupus*) in the Upper Gallatin Canyon of southwest Montana, USA, December through May in the winters of 2003-2006. First I reviewed elk winter diet studies to understand what drives elk foraging behavior. Next I modeled the consequences of diet shifts in grazing and browsing on mass dynamics in wintering female elk. I also developed a new nutritional index, fecal chlorophyll, that I used primarily between winter and spring periods of nutrition as foraging constraints (and costs of antipredator response) would be quite different between these two periods. Specifically, I measured foraging behavior, diet selection, and nutrient balance in wintering elk and monitored daily predation risk as wolves moved naturally, in and out of four creek drainages that formed the primary winter range. Elk showed great sensitivity to fine-scale descriptions of wolf predation risk in nearly every response variable. In particular, adult female elk increased browsing on woody stems, sagebrush, and confers while adult males showed the opposition response and increased grazing on days when wolves were present in the same drainage. This work implies that predator may in fact play a large role in ecosystems including ecosystems where predators were deemed non-influential and bottom up effects important.

CHAPTER 1

INTRODUCTION

The theoretical, experimental and observational study of predation has a long history (Begon *et al.*, 1996). Acts of predation are short-lived but often conspicuous events with obvious consequences for the participants. Because successful acts of predation depend on the death or suppressed growth of prey, predation can be a strong force, guiding predator-prey evolution (Byers, 1997; Geist, 1987; Walsh & Reznick, 2008) and shaping the structure of communities (Estes *et al.*, 1998; Croll *et al.*, 2005). A key focus of predation research is how, and at what magnitude, are species distributions and abundances affected by predators (Sinclair, 1985; Carpenter *et al.*, 1987). Many have focused on the difficult effort of describing and quantifying the numeric impact of predation (Testa, 2004; Valkama *et al.*, 2005; Sinclair *et al.*, 1990; Ebling *et al.*, 1964).

For decades, ecologists have recognized other interactions between predators and prey that are far more common, but less conspicuous, than acts of direct predation (Caro, 2005). The greatest of these interactions are antipredator responses: behaviors or traits that are best explained as adaptations to avoid predation. We can discern two types of antipredator responses that should improve survival: responses to avoid being killed or captured by attacking predators, such as fleeing by elk (MacNulty *et al.*, 2007) or inking by squid (Kicklighter *et al.*, 2007) and responses to avoid detection or selection by non-attacking predators. In regards to the latter response, millennia of natural selection on prey has led to the ability to (1) detect predators, (2) assess vulnerability to predation

from environmental cues, previous experiences, or both, and (3) respond to perceived risk in a way that increases the likelihood of survival. Logic would also suggest that prey that mount antipredator responses only when predators are near would be favored over individuals that always behave as though conditions were risky. This should be true if responses to predators carry costs. Prey individuals that take steps to reduce their risk of predation only when the probability of attack is highest should show higher fitness in a population of either careless conspecifics or pathological responders, as long as the remedial measures do not include fitness costs larger than the gains from reduced predation risk.

The magnitude of the costs of antipredator responses (risk effects) has received increasing attention in the literature (Peacor & Werner, 2001). It is clear that across diverse taxa, the responses of prey to avoid predation come at the expense of other important life-history strategies (Caro, 2005). Quite frequently, the costs of antipredator strategies are paid through altered nutrition acquisition and balance (Anholt & Werner, 1995; Schmitz *et al.*, 1997; Altendorf *et al.*, 2001). Interestingly the magnitude of such costs have been shown to exert equal or even larger effects on prey population dynamics than the numeric effect of direct predation (Pangle *et al.*, 2007; Nelson *et al.*, 2004). This implies that an important influence of predators on prey populations may actually be disguised as a bottom-up effect because predators can (and do) affect nutrient acquisition and balance in prey.

Predators exerting influence on interactions between prey and the environment present some interesting consequences for how we must study predator-prey interactions

and how we interpret the existing literature. The current reasoning for why risk effects have been selected for (i.e., the purpose of incurring costs in the face of predation risk) is to reduce the probability of dying by predation. Therefore, large risk effects might co-occur with small effects of direct predation because those organisms exhibiting greater antipredator responses should be rewarded with decreased rates of attack. If mounting an antipredator response came with costs but did not decrease risk, then the individual displaying such a response would suffer decreased fitness and such a behavior would not be favored by natural selection. Thus, risk effects may be inversely related to direct effects, and in some cases, risk effects are in fact larger than direct effects (Figure 1.1).

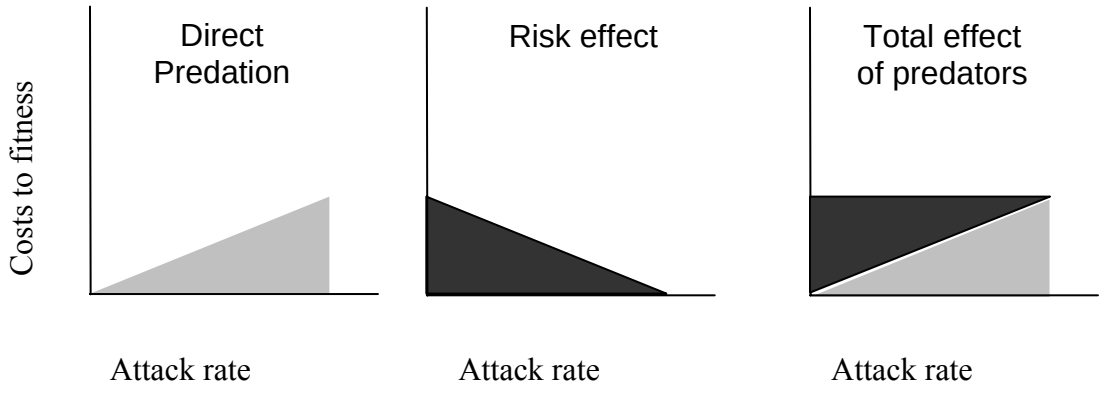


Figure 1.1. Risk effects might be inversely proportional to direct effects if the attack rate is detrimentally affected by antipredator responses. Individuals, populations, or species exhibiting strong antipredator responses with high costs should be rewarded with low predation.

If the total effect of predators is a balance of risk and direct effects that are inversely related, then a logical, but not obvious, condition arises. This pattern suggest that it is possible that some animals (or plants) might be suffering severely depressed survival, growth, and reproduction due to ancestral and current levels of predation risk

even though none or few individuals are ever killed by a predator. Restated, even in prey populations where no evidence of direct predation is found, predators may still be the factor most strongly regulating the prey population. Similarly, in prey populations that experience regular predation, the total effect of predators may be underestimated. It is not safe to assume that important, food-related factors (e.g., resource availability, body reserves, migration, competition) can be considered separately from predation (Mcnamara & Houston, 1987; Werner & Anholt, 1996) even when bottom-up effects are found to be strong, and direct predation is deemed insignificant (Krivan & Schmitz, 2004; Sinclair & Krebs, 2002; Wilmers *et al.*, 2007).

I undertook this study to examine the potential for risk effects to occur in elk (*Cervus elaphus*) in response to predation risk from wolves *(Canis lupus*). To reveal risk effects in a wild population is exceptionally difficult- efforts to minimize predation must be parsed from complex strategies for survival, growth, and reproduction. First, I reviewed the literature on elk winter foraging, as nutrient balance in winter appears extremely important to elk evolution, life history, and population dynamics (Chapter 1). Exploring the effect of various environmental circumstances on diet composition provided insight into the rules elk use for harvesting nutrients, largely in the absence of predation. Next, pooling experimental data from winter feeding trials with captive elk, I explored the consequences for mass dynamics of variation in diet composition (Chapter 2). This analysis confirmed that factors affecting diet selection (more precisely, the balance of grazing and browsing) are very important for winter mass dynamics in elk.

These data, combined with previous findings generated the primary hypothesis I tested in this dissertation.

Creel et al. (2005) found that elk shifted from open grasslands in the absence of wolves to habitats where conifers were more common when wolves were near. My review of winter diets suggested that this habitat shift is likely driving a diet shift towards increased browsing. My modeling of mass dynamics suggested that this diet shift should carry nutritional costs. Specifically, I hypothesized that elk would browse more on days when wolves were present within the same creek drainage. I further hypothesized that this diet shift would alter elk nutrient balance but the direction of this effect (positive or negative) would depend on nutrient balance in the absence of wolves. I tested these hypotheses in a wintering population of elk occupying 3 sites in the Upper Gallatin Canyon, southwestern Montana, USA (Chapters 4, 5 & 6). I simultaneously monitored daily presence or absence of wolves on these three sites while collecting data on elk behavior, nutrition, and environmental conditions. Previous work (Winnie & Creel, 2007; Winnie *et al.*, 2006) also suggested additional hypotheses, namely, that responses to wolves would differ in magnitude between sexes with bulls showing less sensitivity and that snow conditions and plant phenology would also largely affect dietary nutrition. In regards to this last hypothesis, I developed a new fecal index that measured the relative consumption of green biomass by elk (Chapter 3). This index proved useful for defining the date at which forage quality improved due to the arrival of warmer, longer days, but importantly this index was sensitive to morphological and behavioral constraints that naturally affect the elk's ability to exploit new growth.

CHAPTER 2

A REVIEW OF ENVIRONMENTAL FACTORS AFFECTING ELK WINTER DIETS

Abstract

Decades of research have produced substantial data on elk (*Cervus elaphus*) diets in winter, when foraging conditions are most likely to affect population dynamics. Using data from 72 studies conducted in western North America between 1938 and 2002, we collated data on elk diets and environmental variables. We used these data to quantify diet selection by elk and to test whether variation in elk diets is associated with habitat type, winter severity, period of winter, human hunting, and study method. Graminoids (grasses and grass-like plants such as sedges) dominated elk diets and consistently occurred at a higher proportion in the diet than in elk foraging habitats, indicating preference. Forbs commonly made up $\leq$5% of the diet, with no evidence for preference; we conclude that forb use is largely incidental to grazing for graminoids. Browse was consumed in proportion to its availability, implying that the amount of browse in the diet was primarily determined by habitat use rather than selection. Comparing the diets of elk and sympatric ruminants, elk consistently selected graminoids more strongly than sympatric ruminants with the exception of bison (*Bison bison*), suggesting that elk are not environmentally forced to adopt the graminoid-biased diet that they normally select. The proportion of open meadows and grasslands on winter ranges was strongly and positively associated with graminoid consumption by elk. The proportion of graminoids in the diet was significantly lower in elk experiencing severe winter conditions or predation risk

from human hunting. The period of winter (early, middle, and late) had only small effects on elk diets, as did the method by which the diet was determined. Overall, variation in elk diets is well explained by a consistent tendency to select graminoids if available, modified by winter habitat type, predation risk and winter severity, which can constrain habitat selection and access to grazing opportunities. To fully understand variation in foraging behavior, biologists should recognize these broad patterns when interpreting resource selection data. Managers should recognize that inconspicuous behavioral responses to environmental stimuli can alter the diet in ways that probably carry nutritional consequences.

Introduction

There have been many descriptive studies of the foraging behavior and diets of herbivores, but a full understanding of foraging ecology requires us to identify how and why diets are altered by extrinsic and intrinsic factors (Owen-Smith & Novellie, 1982; Hobbs *et al.*, 1981). Extrinsic factors include the quality, abundance and distribution of forages, predation risk, climate, and geophysical characteristics. Collectively, these determine the range of potential diets available on the landscape and affect the costs of obtaining a given diet (Sinclair and Arcese 1995, Hanley 1997). Intrinsic factors include skull morphology and dentition, digestive physiology, and nutrient requirements (Hanley 1982, Hoffman 1989). Collectively, these affect the harvesting costs and nutritional benefits of a given diet, among the potential diets defined by extrinsic factors.

When using terms such as ‘diet’ and ‘nutrition,’ confusion can arise if it is not clear whether one is referring to foraging behaviors, the diets obtained by those behaviors, or the physiological implications for the animal. Throughout this paper, we use diet and dietary to refer to the botanical composition of the forages selected; we use nutrition to refer to the nutrients of a given diet; and we use nutritional condition or condition to refer to the animal’s nutrient balance or health.

For ruminants such as elk (*Cervus elaphus*), a primary foraging decision is whether to browse (on woody plants) or graze (on forbs and graminoids; i.e., grasses, sedges and rushes). The decision to graze or browse may be closely related to metabolic demands for protein and energy (Helwig 1957, Van Soest 1982, Hoffman 1989), because grass and browse differ in digestibility, protein content, and available energy (Cook 1972). Consequently, the optimal balance of grazing and browsing depends on an individual’s current stores of energy and protein, and the trade-off in nutrient harvest and safety when grazing or browsing under predation risk (Pierce et al. 2004). Beginning in the fall, the quality (both protein and energy content) of all foods on elk winter ranges decreases rapidly with the cessation of growth and the onset of weathering. Graminoid quality continues to decline throughout the winter, whereas browse typically retains higher quality, further complicating diet choices (Cook 1972).

In response to winter conditions, elk forage primarily around dawn and dusk and total time foraging is reduced (Mackie 1965, Craighead et al. 1973, Lieb 1981, Green and Bear 1990). Forage intake is reduced and elk retain forage longer, probably to increase the extent of digestion of poor quality foods (Spalinger et al. 1986; Case 1994; Jiang and

Hudson 1996*a*, *b*). When elk nutrition shows a negative protein or energy balance, body tissues are metabolized to meet nutritional needs (Case 1994). These findings partly explain why elk winter nutrition is often severely depressed (Houston 1982, Delguidice et al. 1991), metabolic activity declines (Lieb 1981), and elk commonly lose weight throughout the winter (Jiang and Hudson 1994, 1996*b*). Thus, trade-offs between browsing and grazing are likely to influence the nutrition and condition of wintering elk, with direct ramifications for population dynamics (White 1983, Illius and Gordon 1990, Parker et al. 1999, Delguidice et al. 2001, Cook et al. 2004).

Most studies of food habits are conducted in a single place and time, and consequently provide little power to test how diets are altered by extrinsic factors such as predation risk, habitat type or winter conditions (Hanley 1997). In this review, we compile data on elk diets from 72 studies distributed across western North America (Fig. 1) over a period of 64 years, to provide a broad description of elk winter diets. We compare the diets of elk and sympatric ruminants to test whether elk were environmentally constrained to adopt the diets that they selected. We then test whether variation in elk diets is associated with variation in several factors that have been hypothesized to affect ungulate foraging behavior, namely habitat type, winter severity, period of winter, and predation risk. Finally, we consider whether our results are affected by differences in the methods of the original studies we summarized. Below, we explain the logic underlying each of the hypotheses that we tested.

Winter Range Habitat Type

Elk in western North America commonly move to low elevations in winter to avoid deep snow that impedes movement and access to plants, particularly grasses with low growth forms (Leege and Hickey 1977, Sweeney and Sweeney 1984). This strategy often yields winter ranges in open foothills and grasslands, in contrast to summer ranges in higher and more wooded areas (Houston 1982, Morgantini and Hudson 1988). For some populations, open areas are not available or barriers impede migration, and the winter ranges of these herds often fall in timbered areas (Hunt 1979, Morgantini and Bruns 1984).

This variation among herds in winter range habitats alters the availability of grazing and browsing opportunities and thus might be expected to affect the diet. Alternatively, elk may alter foraging behavior to compensate for variation in habitat types so that the diet is not affected by variation in winter range habitats. Although preferences for certain forages are common in ruminants, diets are often closely related to the relative abundance of different foods (Galt et al. 1982, Ferguson et al 2001). Both absolute and relative abundance of forages have been shown to affect diet composition (Westoby 1974). Here, we tested the hypothesis that diets on more forested winter ranges would include a higher proportion of browse.

Severity of Winter Conditions

Beyond the broad effects of habitat type discussed above, elk diets may be influenced by smaller-scale decisions about local feeding sites. In winter, elk tend to choose locally open areas (grasslands, meadows, and shrub steppe) in preference to other

foraging sites (Singer 1979, Irwin and Peek 1983, Telfer 1994, Baty 1995, Unsworth et al. 1998) but snow depth or hardness can impede cratering (moving snow with the hooves and muzzle to expose forage) that is necessary to feed in open areas in winter (Knight 1970, Houston 1982, Jenkins and Wright 1987). Elk may also select feeding sites in dense vegetation or timber stands to lessen thermoregulatory demands (Beall 1974) or for ease of movement during snow cover (Knight 1970, Peek et al 1982). Thus we hypothesized that elk diets may be related to the severity of winter.

Methods of Determining Diets

Several protocols are commonly used to determine diet composition in ungulates, and the studies we compiled varied in their methodology. Because different methods may have different biases, we considered methodological differences in our pooled data. Three major methods have been used to quantify elk diets: 1) quantifying plant-use by direct observation (Cowan 1947) or by investigating feeding sites (Rouse 1957), 2) rumen content analysis that tallies the frequency (Denio 1938*a*) or volume (Greer 1958) of plant types, and 3) microhistological analyses of plant fragments in fecal pellets (Holsworth 1960, Sparks and Malachek 1968). Plant use methods can be subjective, creating the possibility for poorly understood procedural biases. Because rumen diet estimates are often based on dead individuals, sampling bias is likely. Fecal analysis differs from both rumen and plant use methods because fecal estimates are affected by foraging decisions made over days, rather than hours. We tested whether differences in methods were associated with observed variation in elk diets, primarily to test whether the apparent effects of other factors might be methodological artifacts.

Predation Risk (Human Hunting)

Animals respond to increases in predation risk in a variety of ways that often include increased vigilance, decreased foraging, and shifts into habitats that reduce rates of detection or attack by predators (Lima & Dill 1990). In elk, predation risk from wolves is associated with a reduction in the proportion of time spent foraging and an increase in the use of the timbered areas (Creel et al., 2005, Winnie and Creel *in press*). Hunting by humans is also associated with reduced use of grasslands and increased use of forests (Lyon 1979; Morgantini and Hudson 1980, 1985; Peek et al. 1982). Similarly, human disturbance (other than hunting) causes significant behavioral and physiological responses by elk, including movement, displacement (Edge and Marcum 1985, Conner et al. 2001), changes in group size, habitat shifts (Coop 1973, Beall 1974, Millspaugh et al. 2000), and glucocorticoid stress responses (Creel et al. 2002). Consequently, we hypothesized that elk diets might show an increase in browsing in herds exposed to human hunting. Most authors did not describe the existence of other predators on winter ranges and this limitation prevented us from estimating the true effect size of predation risk on elk diets.

Period of Winter

As mentioned above, woody plants and graminoids both decline in protein and energy content as winter proceeds, but this process is more rapid and less asymptotic in graminoids. Thus, the relative utility of grazing and browsing to meet protein and energy demands may change as winter proceeds. In addition to changes in the plants, the

nutritional status of the elk changes as winter weight loss proceeds (Delguidice 1991). For example, when fat stores have been eliminated and nitrogen balance becomes negative, increased catabolism of muscle affects nitrogen cycling in ruminants (Case 1994). For these reasons, we hypothesized that elk diets may shift as winter proceeds.

Methods

We tabulated data from studies of elk diets primarily using electronic databases and indexes (see Appendix A for a summary of the original studies). We searched state and federal wildlife department Pittman-Robertson reports and other publications using the U. S. Fish and Wildlife Reference Service (USFWS 2003) and the U. S. Department of Agriculture Forest Service Library Catalog (USDA 2003). We searched university libraries and theses using online electronic library catalogs at major universities in the western United States and Canada. For every study we located, we searched reference citations to identify earlier studies.

We restricted the data to winter studies of elk populations that experience significant energy or nutrient restriction due to plant dormancy or snow cover because these are the conditions under which foraging is most closely related to population dynamics (Jedrzejewski et al. 1992, Delguidice et al. 2001, Lubow and Smith 2004). We excluded studies from coastal areas and studies of Tule elk (*C. e. nannodes*) and Roosevelt elk (*C. e. roosevelti*) to avoid genetic or cultural behavioral differences.

We also compiled data on winter habitat use and habitat composition, which allowed a direct comparison of the diet to some measure of forage availability, to test for

selection (Nudds 1980). Some authors simultaneously collected forage cover or biomass data from elk feeding sites while collecting elk diet data. Additionally, for 3 diet studies from Yellowstone National Park, Wyoming and Montana, USA, we located habitat composition data from concurrent elk winter range studies published elsewhere in the literature. For our analysis, we grouped forage items into the same categories as diets (See Dependent Variable – Diet Composition, in this section) and expressed each forage category's availability as a proportion of the total forage available, which summed to 100%. Under this approach, the ratio of use (occurrence in the diet) to availability (occurrence in the habitat) is >1 for foods that are preferred.

Studies that simultaneously collected data for elk and other species allowed us to compare elk diets to those of sympatric white-tailed deer (*Odocoileus virginianus*, $n = 18$ independent diet determinations, see below), mule deer (*O. hemionus,* $n = 72$), moose (*Alces alces*, $n = 11$), bighorn sheep (*Ovis canadensis,* $n = 11$), pronghorn antelope (*Antilocapra Americana*, $n = 3$), and bison (*Bison bison,* $n = 12$). We restricted this analysis to studies of elk and sympatric ruminants using the same winter range, though pairs of data sets were not necessarily from a single study and, therefore, not necessarily concurrent (see Fig. 4 for sources).

Dependent Variable - Diet Composition

Our goal was not to evaluate species-specific plant preferences, which have been well reviewed by Kufeld (1973) and Cook (2002). Rather, we wanted to address the use of major plant forms, so we grouped plants into 3 categories: graminoids (grasses, sedges, and rushes), forbs (flowering plants), and browse (half shrubs, shrubs, and trees). These

categories distinguish groups of plants with similar nutritional value and similar harvesting constraints, and the use of broad categories allowed us to include a large number of studies despite variation in methods among studies.

Some studies reported a diet whose components summed to less than 100%, usually because the authors left relatively uncommon food species unreported. In these cases, we corrected each percentage by restating it as the percent of the reported diet (so that each diet summed to 100%). This adjustment was usually small, and assumes that the unreported portion of the diet was composed of the same balance of graminoids, forbs, and browse as the reported portion.

The original studies varied widely in the amount of data compiled and in their methods of aggregating data to calculate a diet estimate. When authors performed several diet determinations on independent fecal or rumen samples but reported a single averaged diet estimate, we weighted the data point by the number of independent determinations. We used these weighted data in all ANOVAs. This weighting causes studies with few diet determinations to have less effect on the results of ANOVAs and *t*-tests. We did not use weights to account for pooling of individual fecal or rumen samples before determining a diet composition. We did this to prevent the results from being heavily influenced by a few studies that aggregated hundreds of samples per diet determination. Furthermore, we did not use weights for diet estimates based on plant-use methods.

We also restricted the influence of large studies by restricting weights to be ≤ 10, (i.e., studies with more than 10 diet determinations received the maximum weight of 10,

and had as much influence as 10 observations of the same value). We set the limit on 10 because the amount of information gained for a given set of conditions asymptotically approaches zero after the number of aggregated diet estimates exceeds ten. We arrived at this maximum allowable weight using the equation $N = 4 \times SD^2 / SE^2$ from Hanson and Graybill (1956). We set the acceptable standard error for our estimates of diet proportions at 10%. Our estimate of the true standard deviation in diet percentages within a population with relatively constant conditions (SD = 15.7) came from an unpublished data set of diet estimates from 69 rumens collected during 4 successive, normal winters (Dec-Feb 1972, 1973, 1974, 1975) in the Gallatin River drainage in southwestern Montana, USA. The variability in these samples and the equation above suggest that approximately 10 samples (9.87) would estimate diet proportions within 10% of the actual mean with 95% confidence.

Independent Variables

Winter Range Habitat Type: For each study, we categorized the winter range that elk used during the study period as open or closed, based upon descriptions by the authors. This variable describes the availability of protective cover on winter ranges in the form of tall shrubs and trees (Peek et al. 1982, Underwood 1982). When possible, we defined winter ranges as closed when open areas (meadows, parks, and ridges) were less than 25% of the total winter range. We could not apply this criterion when a quantitative description of the habitat was not included in the original study. In these cases we

categorized winter range on the basis of the author's original categorization or description.

We classified all montane-desert scrub ranges (commonly pinyon-pine/juniper habitats) as closed. This may seem counterintuitive given the relatively open feel of these ranges compared to conifer forest, but the generally high density of shrubs and small trees in these habitats supports a designation as closed. This rule only applied to 3 of the 72 studies, so our results were probably unaffected by this decision.

Severity of Winter Conditions: For each diet estimate we categorized the winter conditions during the analyses as hard or normal based upon the authors' description. We assumed the winter was normal if the author did not state otherwise. Because few studies explicitly reported mild conditions, we could not explicitly distinguish mild winters from normal winters.

Methods of Determining Diets: Elk diets have traditionally been determined by 3 methods: plant-use, rumen content analysis, and microhistological analysis of fecal fragments. Because differences among methods could create methodological artifacts in the data, we included method of diet estimation as a factor with three levels (fecal, rumen, or plant-use) in our analysis.

Predation Risk (Human Hunting): Most of the original studies noted whether the elk population was concurrently hunted by humans. We included the occurrence of hunting during the period for which diets were estimated as a categorical independent

variable with two levels (yes or no) in the ANOVA. Data limitations did not let us test for effects of predation other than human hunting.

Period of Winter: To test whether the progression of winter influences diet composition, we included the period of winter at the time of sampling as a categorical independent variable with three levels (*early* = Nov-Dec, *middle* = Jan-Feb, *late* = Mar-Apr). Some studies contributed data to all periods; others contributed to only 1 or 2 periods.

Statistical Analysis

We used factorial ANOVA to test main effects and interaction terms for effects on the diet. Because forbs formed a minor portion of all diets, we combined forbs and graminoids into a single dependent variable, the proportion of herbage in the diet. Because the proportions of herbage and browse in the diet summed to 100%, tests of factors that alter the proportion of herbage also reflect changes in browsing, and dual tests would be redundant. The frequency distribution for the proportion of herbage in the diet showed a strong negative skew that we corrected by cubing and confirming normality through residual plots (Kutner et al. 2005). We implemented the weighting procedures described above by applying weights in the factorial ANOVA module of STATISTICA (Statsoft Inc, Tulsa, Oklahoma, USA). We could not test all factors in a single ANOVA because some combinations of factor levels were not included in the data set, so the necessary variance components could not be estimated. Consequently, tests of some factors (period of winter, hunting, and methods) and interactions were restricted to

data from open ranges in normal winters because this combination included more data than any other.

Results

Broad Diet Patterns and Comparison with Sympatric Ruminants

Seventy-two original studies (Appendix A) conducted across western North America (Fig. 2.1) provided diet estimates for our analyses. These studies reported 305 diet estimates based on ≥1,483 distinct determinations, with some diet estimates coming from a single individual and others coming from groups (largest group ≥110 elk). Applying our weighting rules yielded a data set of 849 diet determinations, with a mean of 2.8 independent determinations per diet.

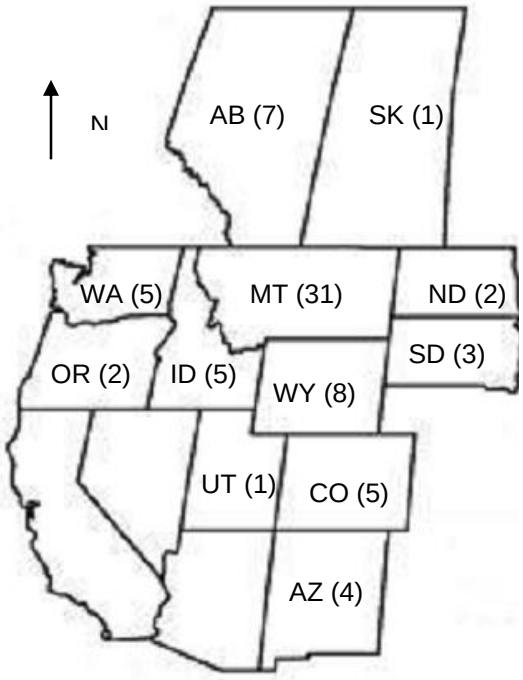


Figure 2.1. Spatial distribution of the 72 elk winter diet studies that we used in analyses. Total sums to 74 because 2 studies by Denio (1938*a*, *b*) provided data from 4 states.

Elk diets were usually dominated by graminoids (Fig. 2.2). Overall, graminoids occurred nearly twice as often in elk diets than forbs and browse combined (paired t = 16.348, df = 848, $P < 0.001$). Browse was selected more frequently than forbs (t = 23.815, df = 790, $P < 0.001$). Forbs were almost always a minor part of the diet, accounting for less than 10% of the diet in 84% of cases. Although these basic patterns were clear, the diets of some elk populations in western North America showed substantial departures from the typical pattern (Fig. 2.2, and note ranges in Table 2.1).

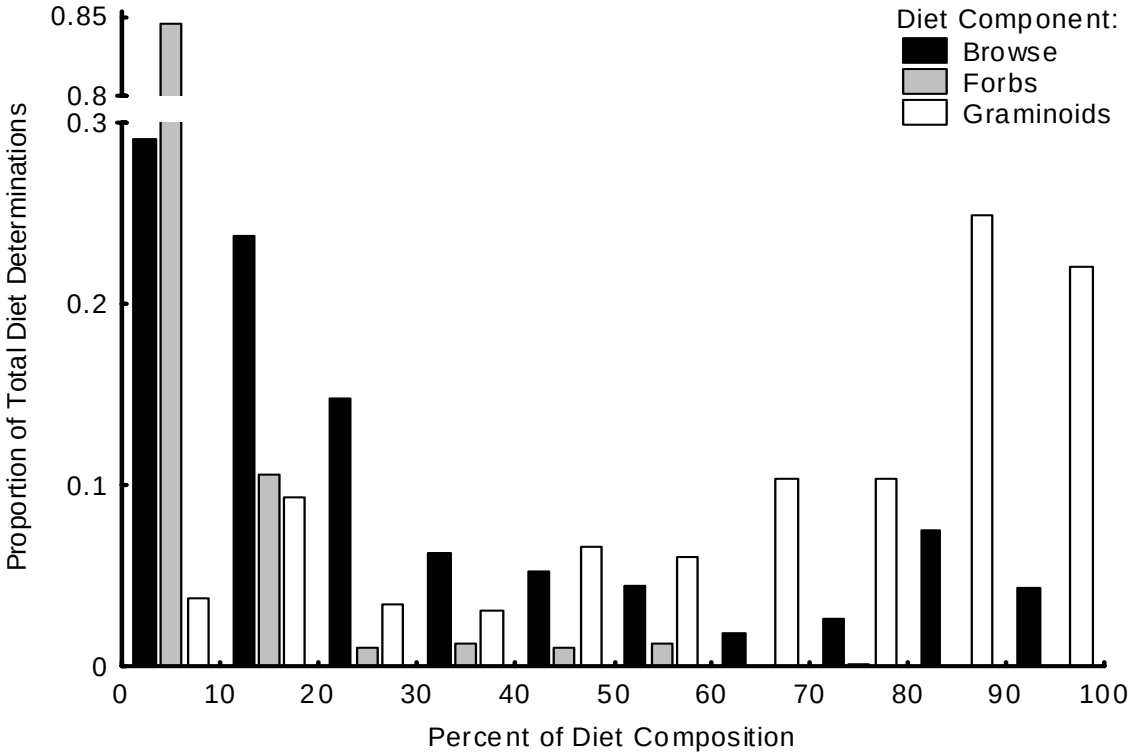


Figure 2.2. Frequency distributions for elk winter diet composition in 72 studies from western North America. Elk winter diets vary widely in the proportions of graminoids and browse but not forbs. Graminoids dominate most winter elk diets, whereas browse and forbs typically occur at low levels.

Table 2.1. Broad composition of elk (*Cervus elaphus*) diets from 72 winter studies in western North America 1938–2001.

Forage type	No. of estimates	Mean % of diet[1]	Range	SD
Graminoid	849	65.8	2.8–100.0	28.1
Forb	792	5.6	0.0–70.0	9.4
Browse	848	29.1	0.0–92.5	27.7

[1] We weighted means by the number of independent diet estimates.

In 14 of the 72 studies, the original authors provided a diet estimate paired with data on habitat composition; additionally, we located the corresponding habitat data for 3 more studies that originally provided only diet estimates. These 17 studies allowed us a broad comparison of use vs. availability. Overall, proportions of forages in the diet were strongly correlated with proportions available at feeding sites (Fig. 2.3) for graminoids ($r = 0.80$, $F = 27.215$, $n = 17$, $P < 0.001$) and browse ($r = 0.79$, $F = 24.314$, $n = 17$, $P < 0.001$) but not forbs ($r = 0.16$, $F = 0.400$, $n = 17$, $P = 0.537$). Superimposed on this pattern, graminoids were always more common in the diet than expected on the basis of availability in the habitat (Fig. 2.3: $t = 3.54$, $n = 17$, $P < 0.001$). Graminoids made up >90% of the diet when they were approximately 80% of the forage available at feeding sites, but a 3-fold decrease in the availability of graminoids resulted in only a 10% decrease in the proportion of graminoids in the diet. Forbs occurred in the diet less frequently than expected on the basis of availability ($t = –4.94$, $n = 17$, $P < 0.001$). Browse use did not detectably differ from random expectation ($t = 1.12$, $n = 17$, $P = 0.14$), though elk tended to under-select browse when it was common (Fig. 2.3).

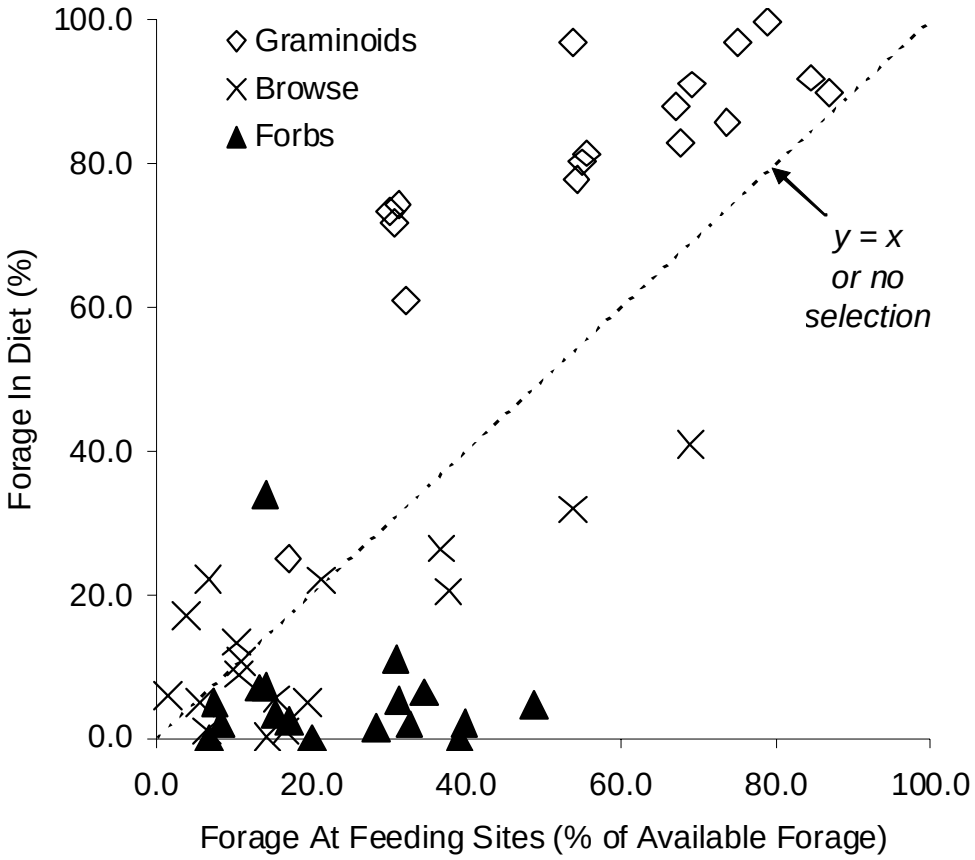


Figure 2.3. The relationship between elk winter diet composition and habitat forage composition. Dashed line represents the relationship in which forages occur as commonly in the diet as in the total available forage (no selection). Graminoids always occurred at higher % in the diet than in the plant community, and graminoids were always selected more strongly than either forbs or browse at similar frequencies of occurrence in the plant community. Forbs occurred less frequently in the diet than in the plant community in all but 1 case. Data from Morris and Schwartz 1957, Picton 1960, Mackie 1965, Stevens 1965, Eustace 1967, Constan 1968, Knight 1968, Greer 1970 (vegetation data from Houston 1982), Cada 1973, Claar 1973, Compton 1974, Cada 1976, Rowland 1981, Kasworm et al. 1984, Baty 1995, Mehus 1995 (vegetation data from Keating et al 1985), Delguidice 2001 (vegetation data from Dawes 1998).

Forty studies allowed a comparison between the diets of elk and a sympatric ruminant (Appendix A). Compared with other ruminants on the same winter range, elk always consumed a higher proportion of graminoids on winter ranges than antelope, bighorn sheep (with 2 exceptions), moose, mule deer, and white-tailed deer, but elk never consumed a higher proportion than sympatric bison (Fig. 2.4, Table 2.2).

Effects of Range Type and Winter Severity

Winter diets were related to range type and winter conditions (Fig. 2.5). Under normal winter conditions the proportion of herbage that elk consumed on open ranges (mean and SD, 85.6% $\pm$ 11.2, $n = 582$) was double that on closed ranges (39.2% $\pm$ 19.6, $F = 83.96$, $n = 86$, $P < 0.001$). It would seem that grazing on open winter ranges is less constrained then on closed winter ranges. This suggests that elk exploit open grasslands when they are freely available.

During hard winter conditions, occurrence of herbage in the diet was less on open ranges (39.6% $\pm$ 28.4%) and closed ranges (13.3% $\pm$ 5.1%) when compared to normal winter conditions ($t = 25.218$, $n = 176$, $P < 0.001$ and $t = 2.943$, $n = 91$, $P = 0.004$, respectively). We found a strong interactive effect between range type and winter conditions: comparing herbage occurrence in the diet during normal and hard winter conditions, there was a smaller absolute difference (25.9% vs. 46%) but a larger relative difference (66.1% vs. 53.7%) between elk from closed and open ranges, respectively, ($F = 14.246$, df = 845, $P < 0.001$).

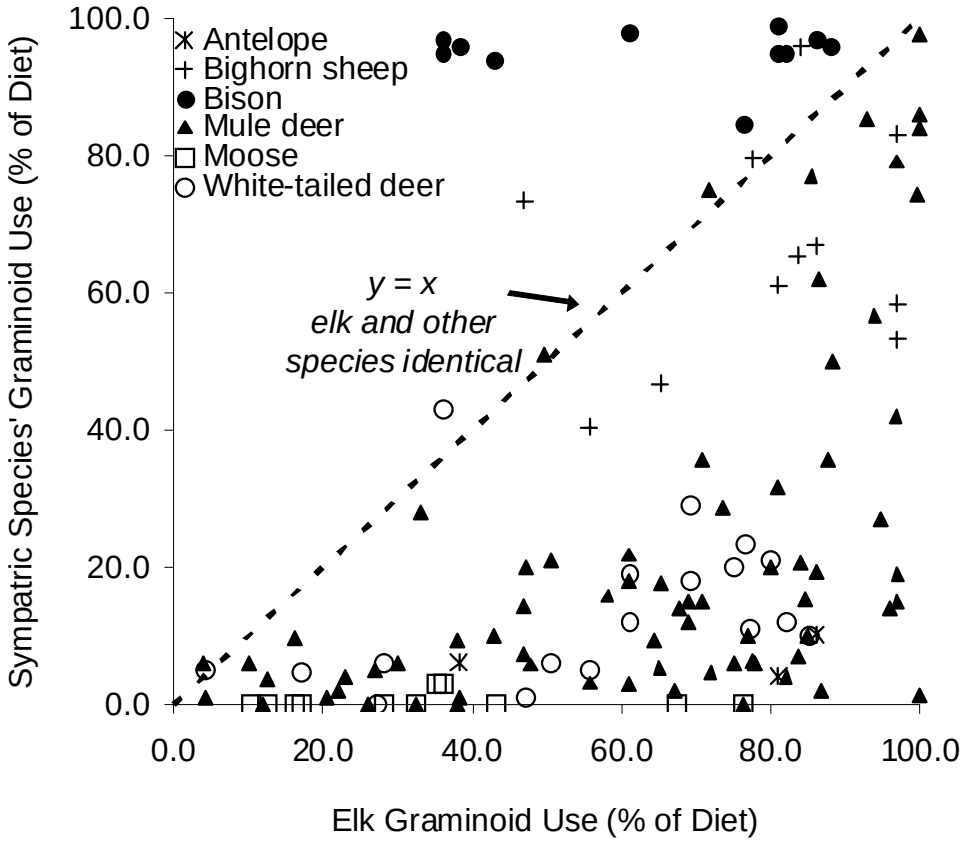


Figure 2.4. Quantitative comparison of winter diets of elk and sympatric ruminants on elk winter ranges. We restricted data to studies of elk and a sympatric ruminant using the same winter range: pronghorn antelope (n = 3), bighorn sheep (n = 11), bison (n = 12), mule deer (n = 72), moose (n = 11), and white-tailed deer (n = 18). Bison were the only ruminant that always selected graminoids more strongly than elk. Bighorn sheep selected graminoids more strongly than elk in 3 of 11 cases. Elk consistently consumed a higher proportion of graminoids than sympatric antelope, bighorn sheep, mule deer, moose, and white-tailed deer. From Denio 1938*a*, *b*; Cowan 1947; Morris and Schwartz 1957; Knowlton 1959; Holsworth 1960; Peek 1963; Mackie 1965; Schallenberger 1966; Constan 1968; Greer et al 1970; Oldemeyer 1971; Claar 1973; Meagher 1973; Compton 1974; Hansen and Reid 1975; Cairns 1976; Komberec 1976; Short et al. 1977; Singer 1979; Skovlin and Vavra 1979; Telfer and Cairns 1979; Barmore 1980; Kvale 1981; MacCracken and Jansen 1981; Keating 1982; Wydeven and Dahlgren 1983; Kasworm 1984; Wydeven and Dahlgren 1985; Jenkins and Wright 1987; Sullivan 1988; Mower and Smith 1989; Vore 1990; Singer and Norland 1994; Telfer 1994; Baty 1995; Mehus 1995; Wright and Kelsey 1997; Delguidice et al. 2001; Tyers 2003.

Table 2.2. Mean percent grass and forbs in winter diets of elk (*Cervus elaphus*) and sympatric ruminants summarized from 40 studies in western North America.

Sympatric ruminant	*n*	Diet	Elk diet	*t*	*P*
Antelope	3	9.7	86.4	2.11	0.051
Bighorn sheep	11	75.3	83.7	1.49	0.077
Bison	12	97.4	71.5	-4.22	< 0.001
Moose	11	0.9	36.4	5.13	<0.001
Mule deer	72	35.0	71.6	8.53	<0.001
White-tailed deer	18	37.6	64.5	3.30	0.001

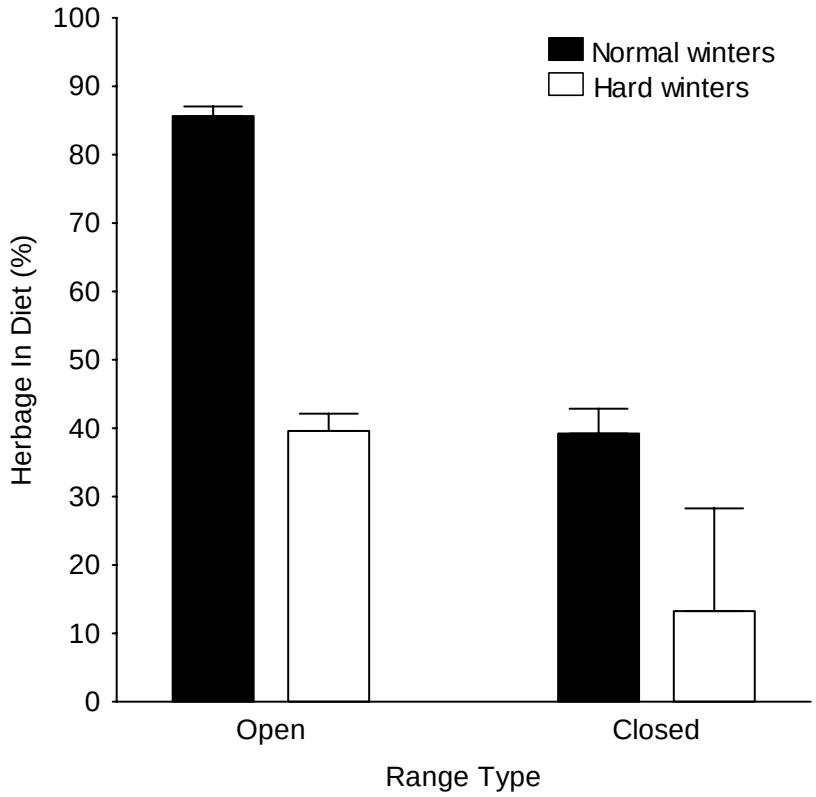


Figure 2.5. Effects of range type and winter conditions on elk diets from 72 studies in western North America. Elk occupying open winter ranges consume more herbage, primarily grass, than elk on closed ranges (heavily forested regions or desert scrub). During periods of normal winter weather, herbage consumption is higher than during periods of unusually heavy snow. Bars show weighted means and 95% confidence intervals. Few data were available for hard winters on closed ranges, widening the confidence interval.

Effects of Study Methods, Hunting, and Period of Winter

Because some combinations of factor levels have not been studied, the following results are from factorial ANOVA using data only from open ranges with normal winter severity (see Methods). These conditions apply to most elk populations in most years.

Methods of Determining Diets: The original studies (Appendix A) used 3 major methods to determine elk diets (microhistological analysis of feces, macroscopic analysis of rumen contents, and direct observations of plant-use at feeding sites). Overall, differences among methods in estimated diets approached statistical significance (Table 2.3), but rumen and fecal estimates (which comprised the majority of the available data) were very similar ($t = 0.112$, df = 567, $P = 0.911$). Variation amongst methods was produced primarily by plant-use estimates, which yielded slightly higher estimates of herbage use when compared to rumen ($t = 2.186$, df = 408, $P = 0.029$) or fecal estimates ($t = 2.463$, df = 183, $P = 0.015$).

To further test for methodological differences, we compared 4 studies from a single elk population. Picton (1960) and Knight (1968) estimated the winter diet of the Sun River elk herd in central Montana with plant-use methods whereas Greer (1960) and Casagranda (1958) estimated winter diets of the same herd with rumen analyses. All diet estimates were collected over a period of 5 winters (1958-1962). Diet compositions based on plant use or rumen analysis were similar for estimates of graminoids ($t = -0.35$, $n = 11$, $P = 0.73$), forbs ($t = 0.66$, $n = 11$, $P = 0.52$), and browse ($t = -0.17$, $n = 11$, $P = 0.87$).

Table 2.3. Factors affecting percent herbage[1] in winter diets of elk on open winter ranges in western North America during normal winter conditions.

Effect	Level	*n*	*x*	SD	*F*	*P*
Method	All	582			2.62	0.074
	Fecal	172	86.0	9.1		
	Plant-use	13	90.7	13.7		
	Rumen	397	85.3	12.0		
Hunting	All	582			35.62	<0.001
	Yes	283	82.8	12.6		
	No	299	88.4	9.0		
Period	All	580			12.65	<0.001
	Early	194	82.6	11.6		
	Middle	240	85.3	13.1		
	Late	146	87.5	11.3		
Method × hunting	All	569			3.04	0.082
	Fecal*yes	12	74.5	6.9		
	Fecal*no	160	86.9	8.7		
	Rumen*yes	271	83.1	12.7		
	Rumen*no	126	89.9	8.6		
Period × hunting	All	580			1.13	0.323
Period × method	All[2]	567			0.52	0.594
Hunting × period × method	All[3]	429			0.98	0.323

[1] Graminoids and forbs (%)
[2] Due to limitations in the data, diets estimated from plant use were not included.
[3] Due to limitations in the data, diets collected in late winter were not included.

Predation Risk (Human Hunting): For elk populations on open ranges in winters of normal severity, populations subject to hunting showed diets with 5.6% less herbage than unhunted populations (82.8% vs. 88.4%, df = 580, P < 0.001, Table 2.3). Although this estimated difference is small, it represents a 40% increase in the amount of browsing (11.6% *vs*. 17.2%), and browse dominated the diet in some hunted elk populations but never unhunted populations (Fig. 2.6).



Figure 2.6. Elk winter diets on open winter ranges in western North America during normal winters with and without human hunting. Browse was the majority of the diet in some hunted elk herds but never in unhunted elk herds. Midpoint line = mean; box = SD; whisker = range.

Period of Winter: The period of winter had a small but detectable effect on diet estimates, with herbage consumption increasing as winter progressed (Table 2.3). This effect was mostly due to differences between early and middle winter (t = -4.43, df = 432, $P < 0.001$) and early and late winter (t = -4.04, df = 338, $P < 0.001$). There was little difference between middle winter and late winter (t = -0.66, df = 384, $P = 0.506$).

Interactions: Because rumen samples come primarily from dead animals, one might expect an interaction between method of diet estimation and the occurrence of hunting. Both rumen and fecal estimates showed less use of herbage with hunting. The estimated decrease in grazing was larger in fecal studies than in rumen studies, but we did not detect an interaction between the effects of hunting and method of analysis (Table 2.3).

We also detected no significant interaction between hunting and period of winter, or between methods and period of winter. Because of imbalances in the data, we could only test 3-way interactions when we did not consider diet estimates from plant use and late winter. We detected no interaction between the 3 factors on open winter ranges during normal winter conditions (Table 2.3).

Discussion

Despite occupying diverse landscapes and consuming a wide range of winter diets, elk in western North America were predominantly grazers when snow cover or habitat composition did not constrain grazing opportunities (Fig. 2.2). As others have found in captive feeding trials and experiments (Helwig 1957, Hobbs 1979) elk strongly selected graminoids over other forages compared to their availability at feeding sites (Fig. 2.3) and when compared to the diets of sympatric ruminants (Fig. 2.4, Table 2.2).These results suggest that in many situations, high graminoid diets are not the result of environmental or physiological constraints as both larger and smaller sympatric ruminants are capable of highly dissimilar, high browse diets. Together, interspecific comparisons and comparisons of use vs. availability suggest that elk prefer graminoids under a broad range of conditions, which typically leads to a highly graminoid-based diet.

By pooling results from many sites, we could explicitly test whether variation in the diet was associated with variation in winter range characteristics or other factors that often remain constant within individual studies (e.g., presence or absence of human hunting). Although elk consistently select for graminoids, habitat type can limit the

expression of this preference. Consequently, the diets of elk living on heavily timbered winter ranges were substantially less biased toward graminoids (Fig. 2.5), even though graminoids were preferentially selected under all conditions (Fig. 2.3).

The severity of winter conditions also influenced elk diets, probably by constraining the opportunity to graze. Elk experiencing hard winter conditions had diets with less than half the herbage of diets under normal winter conditions for both open and closed ranges. This broad result is congruent with patterns identified by individual studies. Jenkens and Wright (1988) showed strong selection for graminoid-dominated habitats (and avoidance of browse-dominated habitats) when snow depth was low. When snow accumulation restricted the availability of graminoids, these habitat selection patterns reversed, and browse consumption increased (Jenkens and Wright 1987). Skovlin and Vavra (1979) found that browse comprised 74% of the diet after a winter storm deposited >31 cm of snow, though browse formed only 27% of the diet in periods with less snow accumulation. Rouse (1957), Greer (1958, 1970), Lovaas (1963), Vales and Peek (1996), and Delguidice et al. (2001) all showed short term increases in browse consumption (1.5 – 7-fold) during periods with increased snow cover. Our analyses show that such responses can be detected broadly in western elk populations and thus are probably not uncommon. Shifting away from grazing arises at least in part because heavy snowfall reduces the accessibility of herbage simply by burying it, but snowfall is less likely to bury woody vegetation (Jenkins and Wright 1987, 1988).

Hunting by humans on open ranges during normal winters was also associated with an increase in the proportion of browse in the diet, with some herds showing a

substantial behavior response (Fig. 2.6). Our analysis probably underestimates the effect of hunting on the diet because 1) hunting pressure probably varied among areas and this variation could not be controlled in our analyses, 2) many studies did not allow any determination of hunting conditions and we categorized such data as no hunting, which would act to obscure effects, and 3) elk behavioral responses to predation risk occur on finer temporal and spatial scales than those examined in our analysis (Creel and Winnie 2005). Individual studies confirm that hunting effects can be large. Morgantini and Hudson (1985) examined the impact of hunting on the diet directly and found elk to consume less grass during a winter hunt than before or after the hunt (57.9% vs. 88.8%) and suggested that this diet shift was due to increased use of protective cover (Morgantini and Hudson 1988).

It is perhaps counterintuitive that elk diets reflected the habitats they occupied (Fig. 2.5), yet elk also showed strong selection for grasses when we assessed selection by comparing use to availability in smaller scale feeding patches (Fig. 2.3). We interpret these patterns to mean that elk winter diets are probably the product of a fine-scale preference for graminoid patches within habitats, superimposed on broad scale habitat selection that is based on forage distribution and environmental factors (Wamboldt and McNeal 1987). Rather than simply searching out key plant species to survive the winter, we suggest that elk respond to environmental stimuli such as plant distributions, weather conditions, snow depth, and predation risk when selecting foraging sites or habitats (White and Feller 2001, Winnie et al. *in press*). Then, within a foraging site, elk preferentially consume graminoids until constrained by environmental conditions to

browse or avoid graminoid patches. This behavior would produce the broad-scale (Fig. 2.5) and fine-scale patterns (Fig. 2.3) that we observed, and it would yield a diet that is usually graminoid-dominated but can vary substantially (Figs. 2.2 & 2.3) depending on local conditions. If open ranges, normal winters, and no hunting are accepted as the conditions under which elk diets most closely reflect behavioral preferences (because constraints are weakest under these conditions), then it appears that elk select graminoids almost exclusively in the absence of constraints. Only as conditions restrict elk choice does the proportion of grass in the diet decline (Fig. 2.7).

These findings have important implications for elk nutritional condition, which strongly affects winter survival and subsequent reproduction (Helwig 1957, Thorne et al. 1976). Given strong selection for grasses, elk nutrition and condition in winter depend heavily on the nutritional profile of dormant grasses (Claar 1973, Rowland et al. 1983), namely low protein content and low digestibility (Cook 1972). There is evidence to suggest that foraging behavior that is grazing dominated may not best meet elk nutritional demands. Indeed, Claar (1973) found that, over 3 winters, deer maintained a diet that was 44% higher in crude protein and only 6% lower in crude fiber than diets of sympatric elk by consuming twice as much browse, which was more abundant than grass or forbs on this winter range. Daneke (1980) also noted that elk selected low quality grasses even when browse forages were more abundant. On the other hand, highly browse-biased diets obtained when constraints on grazing are strongest may also have negative nutritional consequences due to deficits in gross energy intake (Helwig 1957, Delguidice 2001).

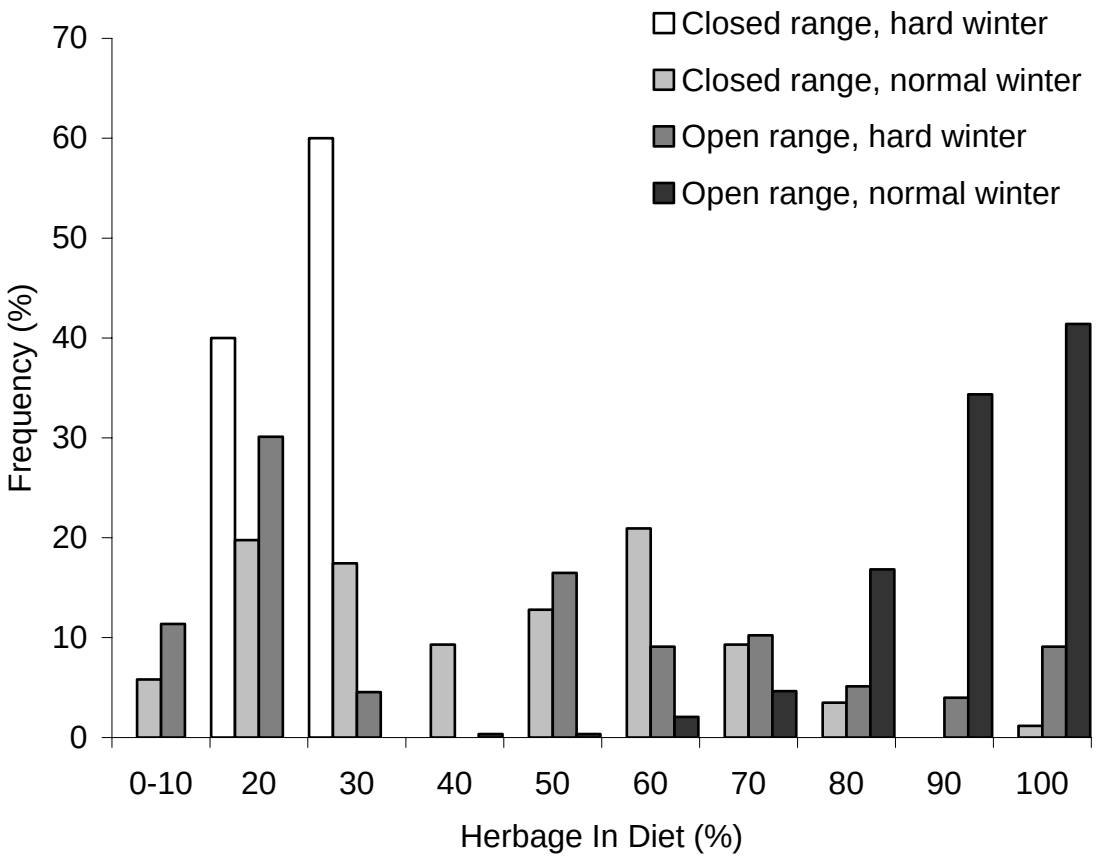


Figure 2.7. Frequency distributions for herbage (graminoids and forbs) consumption with different winter range and weather conditions from 72 winter studies of elk diets in North America. As environmental conditions reduce constraints on grazing, elk respond by increased grazing; the diet is almost entirely composed of grass on open ranges without severe winters.

Management Implications

Biologists should understand these basic patterns in elk winter foraging, especially when interpreting diet estimates or habitat selection data which may indicate over or under-selection for resources when compared to these broad results. Further, the plasticity of the elk diet and its clear response to environmental factors implies nutritional consequences that are poorly understood. Future research should focus on, and managers

should be aware of, the relationships between foraging behavior, reproduction, and survival that may drive population dynamics in an inconspicuous manner. Such inquiry will be relevant to social issues (e.g. crop depredation, supplemental feeding) and scientific concepts (e.g. predator-prey interactions) associated with elk foraging in winter.

Acknowledgements

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CHAPTER 3

THE OPTIMAL BALANCE BETWEEN GRAZING AND BROWSING IN ELK FROM MODELS OF MASS DYNAMICS

Abstract

The factors that favor grazing on herbaceous vegetation or browsing on woody vegetation have received considerable attention in studies of herbivore ecology and evolution. Much of this research has focused on patterns and mechanisms of niche partitioning in the diets of specialized grazers and browsers, but the role of grazing and browsing within intermediate feeders presents unique questions that have received little attention. We explored the role of grass and browse in the diet of an intermediate feeder and the importance of ideally mixed diets for maintenance of body mass. We compiled published data on the mass dynamics of captive elk (*Cervus elaphus*) fed diets of pure grass, pure browse or one of 14 mixed diets in winter feeding trials. Elk lost mass in 29 of 33 feeding trials, up to 22% of initial body weight, similar to wild elk in winter. We used model selection to compare 18 linear models of mass dynamics, considering the linear and quadratic effects of the grass consumption, dietary nitrogen, and intake rate with initial metabolic mass and feeding situation (alone or in pairs) as covariates. The model with the linear and quadratic effects of grass consumption and dietary nitrogen performed best (R^2=0.83). We then simplified this model by incorporating the mean dietary nitrogen found in grass (0.59%) and browse (1.14%) in the wild. Using this simplified function to explore the role of diet composition in wild populations, optimal

diets near 53% grass, 47% browse were predicted to minimize winter mass loss. Temporal variation in grazing and browsing and concurrence of grass and browse in the digestive tract may be a unique constraint on fitness in intermediate feeders that may not always be optimized in wild populations.

Introduction

The composition and quality of the diet has played a central role in theories and empirical studies of herbivore diversity, distributions, abundance, and community interactions (Hofmann & Stewart, 1972; McNaughton & Georgiadis, 1986; Augustine & McNaughton, 1998; Clauss *et al.*, 2003). In this literature, the proportion of the diet that is grazed (herbaceous vegetation) versus that which is browsed (woody plants) has been a central theme. Across taxa, patterns of grazing or browsing correlate strongly with morphology, behavior, and life-history strategies (Jarman, 1974; Janis *et al.*, 2002; Fritz & Loison, 2006). Within ecosystems, the diversity of grazing and browsing herbivores relates to nutrient cycling, primary productivity, and community stability ((McNaughton, 1985; Olff *et al.*, 2002; Martin, 2007). Variation in the physico-chemical properties of grass and woody browse, the spatial distribution of these forages, and the structural diversity of the landscapes where each forage is most common have been strong drivers of herbivore evolution (Janis *et al.*, 2000; Perez-Barberia & Gordon, 2001; Clauss *et al.*, 2003; Fritz & Loison, 2006).

Because browsing and grazing are functionally and nutritionally quite different, specific food-habits have proven particularly useful in models, theories, and explanations

of herbivore ecology and diversity (Clauss *et al.*, 2003; Hofmann & Stewart, 1972). Diet selection by large herbivores is often simplified by describing a species' position on a continuum with pure browsers at one extreme and pure grazers at the other. Intermediate feeders are a catch-all group of herbivores that vary considerably in their ecology, behavior, and biology, surviving somewhere between these two strategies by consuming mixed diets of grass and browse (Hofmann & Stewart, 1972). Intermediate feeders generally do not take 'mixed' bites of grass and browse or even necessarily forage in 'mixed' feeding patches. Instead, the balance of grass and browse in the diet is largely dependent on the balance of decisions to forage in grasslands areas or woodlands ((Hansen & Reid, 1975; Hanley, 1980; Stewart *et al.*, 2002). That is, intermediate feeders seem to lack specific foraging behaviors or 'rules' for allocating bites to browse or grass, and mixed diets are the result of feeding opportunistically while expressing diverse habitat preferences (Morgantini & Hudson, 1985; Singer & Norland, 1994; Christianson & Creel, 2007). This is interesting because these patterns of habitat selection are often driven by factors not directly related to diet (e.g., predation risk, disturbance, or thermoregulation) and can be highly variable (Jarman, 1974; Goldsmith, 1990; Conradt *et al.*, 2000; Creel *et al.*, 2005). Such shifts in habitat use could present unique consequences for diet selection and nutrition in intermediate feeders that have no obvious parallel in obligate grazers or browsers. If a factor such as predation risk causes a change in habitat selection, the diets of intermediate feeders should change dramatically, in comparison to the diets of pure grazers or browsers, and the consequences for nutrition might be considerably different as well. Understanding these differences between

feeding types and the consequences for similar behavioral responses could have important implications for herbivore ecology and evolution. To date, most diet selection models have focused on species at the extremes of the browser-grazer continuum where considerable specialization exists (Belovsky, 1981; Owen-Smith & Novellie, 1982; Blackburn & Kothmann, 1991; Newman *et al.*, 1995). However the often mutually exclusive and habitat-dependent decision to browse or graze faced by intermediate feeders presents a unique and complex set of decisions that have proven difficult to describe and are rarely considered in theoretical or empirical research.

The lack of specialization in mixed feeders suggests they are less efficient than either grazers or browser in harvesting and assimilating grass or browse (Renecker & Hudson, 1990; Wickstrom *et al.*, 1984; Clauss *et al.*, 2003), but they may have traded these benefits to avoid costs that might be associated with exclusive grazing or browsing, such as habitat-specific predation risk or increased sensitivity to the temporal variability in forage abundance (Byers, 1997). Yet, because the broad differences in anatomy, physiology, and behavior between grazers and browsers are due to adaptations to the broad differences between grass and browse, we should expect that intermediate-feeders possess unique adaptations to a specific balance of grazing and browsing. In line with this hypothesis, the location of mixed-feeders on the grazer-browser continuum generally follows the expected pattern based on habitat selection, morphology, and nutrient requirements (Janis *et al.*, 2002), i.e., intermediate feeders with adaptations similar to grazers often graze more than they browse. We should expect that most intermediate feeders are adapted to maximize nutrition when consuming some optimal combination of

grass and browse (Fig. 3.1). Despite this expectation, this assumption is not implicit in many models of herbivore foraging ecology, and we have found no studies in the literature that describe or predict this balance, or the magnitude of the nutritional costs that must be paid by intermediate feeders for deviating from this optimum.

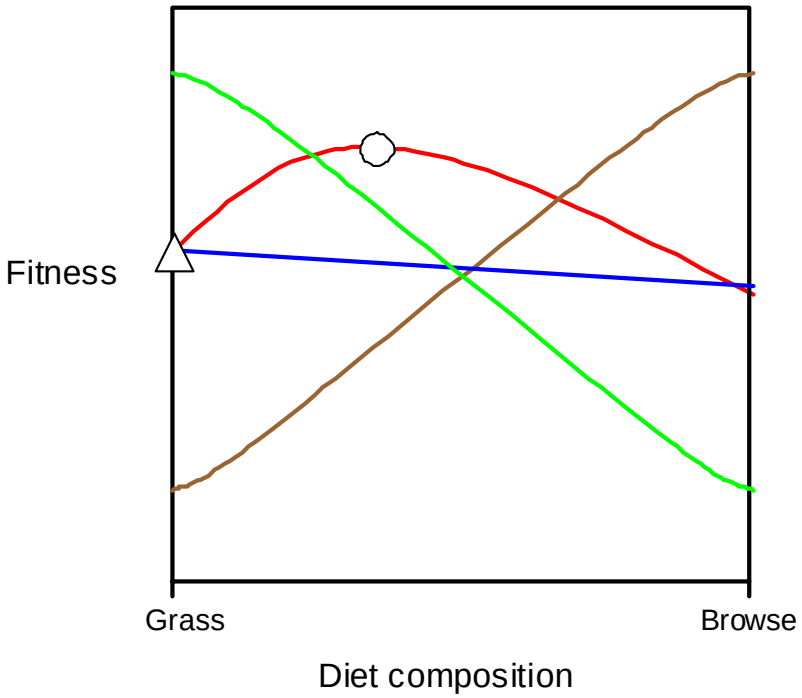


Figure 3.1. Current theories of herbivore evolution focus on grazers (green line) and browsers (brown line) and predict costs for deviations in diet from the respective ends of the browser-grazer continuum. Intermediate feeders (IF) have received less attention but current models implicitly suggest that because IF are not specialized, fitness for pure grass or pure browse diets exists somewhere between the payoff for obligatory grazers and browsers (ends of blue and red lines). However, the fitness payoff for mixed diets in IF is less clear. If a simple linear relationship exists between fitness and diet mixture (blue line), this implies that the optimal diet for a hypothetical IF that performs marginally better under one strategy (slope of the blue line) is no different than that of a specialized herbivore. The optimal diet of such an IF would be 100% grass (triangle). Under such a scenario, adaptations to mixed diets would be selected against, in favor of increased specialization for the optimal strategy and IF could not evolve. Thus, logic suggests that fitness for IF should be maximized as the diet becomes increasingly mixed (red line) and some optimal balance of grazing and browsing should exist (circle).

If a precise balance of grass and browse in the diet is essential for optimizing an intermediate feeder's nutrition, then the periodicity, not just proportionality, of grazing and browsing to achieve that optimal balance must also be important. Many different patterns of foraging can ultimately produce the same diet composition, yet vary widely in the number of alternate bouts of grazing and browsing. The degree of temporal overlap of grass and browse in the digestive tract could also differ considerably between foraging patterns, despite similar diet composition. Empirical data confirm that the frequency of alternating grazing and browsing within a species of intermediate feeder can be highly variable (Gebert & Verheyden-Tixier, 2001; Christianson & Creel, 2007). This variation highlights an important distinction from grazers and browsers when describing the 'average' food habits and their nutritional benefits: some species or populations of intermediate feeder may consume mixed diets of grass and browse almost daily (Hobbs *et al.*, 1981), whereas others may alternate between grazing and browsing on a seasonal basis (Hulbert *et al.*, 2001). The mechanisms that drive this variation are poorly understood and the significance of the time intervals over which the diet becomes 'mixed' is unknown. Surely, given the broad differences in grass and browse, and grazers and browsers, the potential exists for there to be significant nutritional differences between individuals that consume diets that are mixed on the short-term, where grass and browse occur simultaneously in the digestive tract, and long-term mixed diets where similar proportions of grass and browse are consumed but rarely overlap in the digestive tract. Confirming whether temporal variation in the relative intake of grass and browse carries nutritional costs would aid in our understanding of diet selection and the evolution

of feeding strategies. To be sure, the consequences for fitness of a particular diet combination are expected to vary across ecological circumstance but the intrinsic, nutritional consequences of the degree of grazing and browsing, free of harvesting costs, are expected to be one important component of fitness that remains poorly understood in wild herbivores.

North American elk (*Cervus elaphus*) are relatively large members of the Cervidae and are hypothesized to have evolved from a browser ancestor to its present form with adaptations for both grazing and browsing in temperate forests and grasslands (Geist, 2002). The food-habits of elk are most often studied in winter because plant dormancy and snow cover are argued to be strong drivers of herbivore population dynamics (White, 1983; Illius & Gordon, 1990; Stewart *et al.*, 2005). Winter mass dynamics of elk correlate with the birth mass, sex ratio, survival, and growth rates of offspring and the timing of reproduction, (Thorne *et al.*, 1976; Hudson *et al.*, 1991; Jiang & Hudson, 1994; Friedel & Hudson, 1994; Cook *et al.*, 2004a; Stewart *et al.*, 2005) and winter mass loss often leads to death by starvation or prolonged, depressed body condition ((Greer & Howe, 1964; Gates & Hudson, 1981; Singer *et al.*, 1997; Gedir & Hudson, 2000; Cook *et al.*, 2004b). Though clearly important for survival and reproduction, the drivers of winter mass dynamics are poorly understood and difficult to study.

To explore how the balance of grazing and browsing affects the nutrition of this intermediate feeder, we compiled experimental data from feeding trials with adult female elk that varied the proportion of grass and browse in the winter diet. We used these

experimental data to explore the effect of diet composition on body mass as an index of the intrinsic, nutritional consequences for fitness of decisions to graze or browse in the wild. The experimental approach is necessarily limited in its inference to wild herbivore ecology, but allows inferences about causation that can provide useful insight into how nutrition is affected by decisions to graze or browse, and the importance of mechanisms that affect those decisions.

Methods

Raw Data on Body Mass Changes Under Experimental Diets

As part of a review of food habits, we conducted a literature search to summarize winter foraging behavior and nutrition in elk, (Christianson & Creel, 2007). This search identified three unpublished theses (Hungerford, 1952; Geis, 1954; Helwig, 1957) that contained raw data from 43 winter feeding trials where change in body mass was recorded for adult female elk fed diets of pure grass, pure browse or one of 14 mixed diets. The studies used similar experimental designs, all at a facility located on the Blackfoot-Clearwater Game Range in western Montana, USA. Trials were conducted during January and February with forages typically eaten by elk in the Northern Rockies: willow (*Salix sp.*), serviceberry (*Amelanchier alnifolia*), lodgepole pine (*Pinus contorta*), Douglas fir (*Pseudostuga menziesii*), and grass (mainly *Festuca sp., Agropyron sp., Agrostis alba, Phleum pratense,* and *Carex sp.*). Forages were harvested from elk winter ranges during or immediately before the feeding trials. Because trials varied in duration and adult females varied in size, we calculated the daily change in mass as a percent of

initial body mass to allow direct comparisons. Thus daily mass change (% of initial body mass) was the primary dependent variable. We then tabulated the following additional data for each feeding trial.

Diet Composition: The proportions (by mass) of browse and grass in the diet were the variables manipulated in these feeding experiments. The original authors harvested grasses as hay and subsampled to estimate species composition. Harvested hays contained an average of 12.2% forbs by mass and we estimated the grass proportion by subtracting the proportion of incidental forbs. We did not evaluate the role of forbs because 1) forb use was consistently small, 2) forbs are insignificant in the diets of most wintering elk populations (Kufeld, 1973), and 3) forbs appear to be consumed incidentally while grazing (Hobbs, 1979; Christianson & Creel, 2007). Additionally, because grass and browse formed nearly 100% of the diet, they were necessarily inversely related to one another ($r = -0.974$), so we did not directly model the effects of browse consumption on mass dynamics. Any browse effects would be directly inverse of grass effects, and dual tests would be redundant. Consequently, when we interpret statistical tests of grass effects in this paper, the inferences are dependent on browse forming the remainder of the diet, as is true in the wild (Christianson & Creel, 2007; Kufeld, 1973; Cook, 2002).

Metabolic Mass: We considered that some variation in mass loss might be due to allometric variation in metabolic demands, so for each elk we converted body weight at

the beginning of the feeding trial to metabolic mass, $kg^{0.75}$ (Board on Agriculture, 2000), and considered this as a covariate in our analyses.

Dietary Nitrogen: The original authors did not attempt to directly control dietary nitrogen intake but they measured crude protein for each forage type. We estimated total dietary nitrogen for each trial by multiplying each forage's nitrogen content (% crude protein $\cdot$ 6.25^{-1}) by its proportion in the diet and summing the products.

Feeding Situation: The original authors conducted 22 of the 43 trials with paired animals in a single pen and only reported an average intake for both animals, although some elk in pairs had apparently different intakes due to social dominance affecting access to food. In these cases, we averaged the mass loss within pairs to correspond with the averaged intakes, and included a covariate recording whether the animals were fed alone or in pairs to avoid bias in our analyses.

Intake Rate: Animals were offered their feeding rations daily and forage consumption was measured as kg of forage/100 kg of body mass. We considered intake rate might be an important covariate because logic suggests that mass dynamics will be affected by both the type and amount of food.

We excluded all data from 10 trials; three from Hungerford (1952), six from Geis (1954), and one from Helwig (1957) due to the following problems: one elk acquired a bruised nose that impaired foraging, two trials included elk that died during the study, three trials were less than three weeks long, and four trials included elk that did not habituate to confinement. Helwig (1957) reported that ~90% of females were pregnant,

similar to rates found in the wild (Creel *et al.*, 2007b), but pregnancy probably affected mass change little as all trials occurred early in the second trimester when fetal growth rates and energy demands are low (Hudson *et al.*, 2002). Others have identified strong relationships between the diet composition and dietary nitrogen and intake that could confound model design (Cook, 1972; Leslie & Starkey, 1985; Morgantini & Hudson, 1989; Arthun *et al.*, 1992); therefore we searched for correlations in these variables prior to model selection.

Model Selection

We used an information-theoretic approach (Burnham & Anderson, 2002) to identify the best models within a set that considered the effects of diet composition on mass loss. To form our list of candidate models, we focused on three questions. (1) How important is the proportion of a grass and browse in explaining mass dynamics? (2) How important is dietary nitrogen in explaining mass dynamics? (3) Do models that include only the linear effects of grass consumption and dietary nitrogen perform better than models that also consider a quadratic effect? This last question considers that the effect of these diet components could asymptote or change direction as they become more freely available.

Specifically, the five variables and 2 quadratic terms we considered produced 127 potential combinations (without interactions), but we defined 18 models, *a priori*, that most directly addressed our questions (Table 3.1). We first considered a global model containing all parameters: the linear and quadratic effects of the grass

Table 3.1. A summary of the models considered in AIC for mass change in winter feeding trials with elk.

#	Variable Combinations[1]	Comments
1	grass + grass^2 + nitrogen + nitrogen^2 + intake + mass + situation	global model
2	grass + grass^2 + nitrogen + nitrogen^2 + intake	simplified global model, forage and intake
3	nitrogen + nitrogen^2 + intake + mass + alone	global model without considering diet composition
4	grass + grass^2 + intake + mass + alone	global model without considering diet quality
5	grass + grass^2 + nitrogen + nitrogen^2	diet composition and quality only
6	grass + grass^2 + intake	diet composition and intake only
7	grass + grass^2	diet composition
8	nitrogen + nitrogen^2 + intake	diet quality and intake only
9	nitrogen + nitrogen^2	diet quality only
10	grass + nitrogen + intake + mass + situation	global model (linear effects only)
11	grass + nitrogen + intake	simplified global model (linear effects only)
12	grass +nitrogen	linear effects of diet composition and quality
13	grass + intake	linear effects diet of intake
14	nitrogen + intake	linear effects diet quality and intake
15	grass	linear effect of diet composition
16	nitrogen	linear effect of diet quality
17	intake	effect of forage intake
18	intake + mass + situation	all variables unrelated to forage

[1] Variables considered in modeling were grass (% grass in a grass/browse diet), nitrogen (% dietary nitrogen), intake rate (kg forage · 100kg body mass^{-1} · d^{-1}), mass (kg body mass$^{0.75}$), and situation (whether the animal was fed alone or as part of a pair)

proportion of the diet (parameter names are hereafter referred to as *grass* and $grass^2$, respectively), the linear and quadratic effects of dietary nitrogen (*nitrogen* and $nitrogen^2$, respectively), the metabolic body mass at the beginning of the feeding trial (*mass*), the forage intake rate during the feeding trial or the average intake for paired animals (*intake*), and whether the animal was fed alone or as part of a pair (*situation*). From this global model, we considered three less complex models with varying combinations of the linear or quadratic effects of *grass* or *nitrogen* in conjunction with the other three explanatory variables: *intake*, *mass*, and *situation*. Six models contained only *grass* or *nitrogen* parameters and we did not include the quadratic effect unless the linear effect was also included. We also hypothesized that *intake* might affect the performance of these six models more strongly than *mass* or *situation*, so we also considered the same six combinations but with intake added as a parameter. Finally, we considered 2 models with neither *grass* nor *nitrogen* to provide scope to test our primary hypothesis, that models of mass dynamics perform best when variables related to diet composition are included.

Because of the physical, chemical, and ecological differences between conifer browse and deciduous browse, we also considered browse type in our modeling, though in a restricted way. We could not apply a browse type variable to all treatments in the data set (some animals consumed no browse) so we explored the performance of the best models (but not coefficients) identified by AIC model selection by refitting them to a data set containing only those animals that consumed some browse. In this test, we simply compared the performance of the original model to the same model with browse type as

an additional term and examined the independent effects of browse type on mass dynamics with a partial F test.

Results

Experimental Diets

The proportion of the experimental diets composed of grass varied from 0 – 97.2% with 16 different values. Trials ran for 26-68 d (mean ± SD, 42.5 d ± 11.5). Total dietary nitrogen ranged from 0.48 to 1.23% and, due to variation within forage types, dietary nitrogen was not detectably correlated with grass consumption ($R = 0.136$ $P = 0.449$). Average daily intake ranged from 1.5 – 3.2 kg of food per 100 kg body mass, and there was no detectable correlation between intake and grass consumption ($R = 0.229$, $P = 0.201$). Metabolic body mass varied from 50.4 kg to 62.5 kg. As is typical for wintering animals, elk lost mass in 29 of the 33 trials (Fig. 3.2) with an average daily change in body mass of –0.17%. Mass loss decreased as dietary nitrogen increased (ANCOVA $F_{1, 28} = 18.25$, $P < 0.001$, slopes of regressions (Fig. 3.2). This sentence is to add filler space so that the gap at the bottom of the page is not so large. If I had more too say I would have originally said it. This paragraph was not that interesting to begin with. Mass loss also tended to decrease as grass consumption increased, except for diets heavily dominated by grass (ANCOVA $F_{3, 28} = 20.17$, $P< 0.001$, intercepts of regressions, Fig. 3.2).

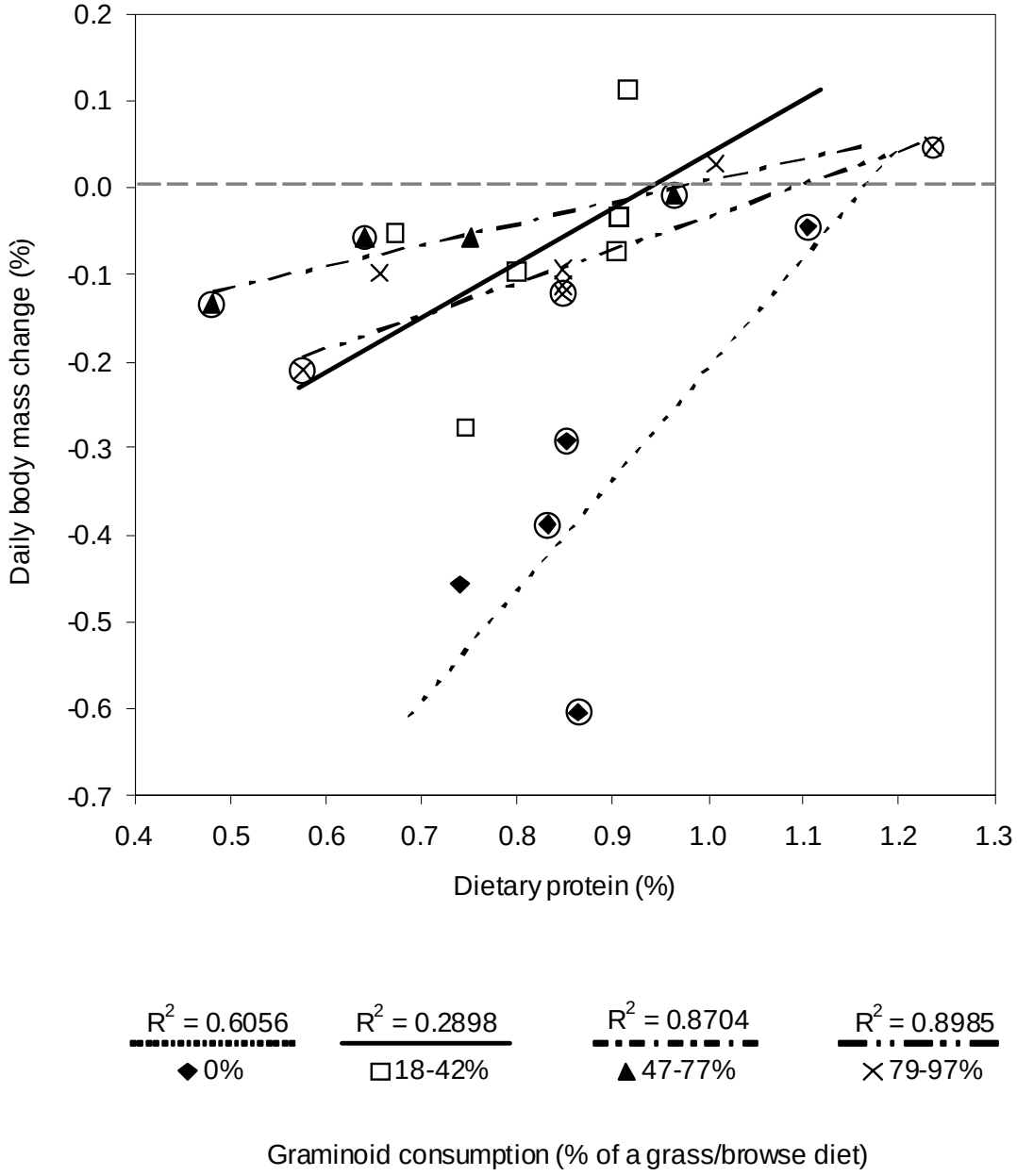


Figure 3.2. Raw data on daily change in body mass as a function of dietary nitrogen and grass consumption from 33 winter feeding trials with adult female elk. Very few winter diets actually prevented mass loss. Here, feeding trials are grouped into four categories based on diet composition as a visual reference, but grass consumption was treated as a continuous variable in modelling. The effect of dietary nitrogen is revealed by the positive slopes of the regressions. The effect of increasing grass consumption is revealed by differences in the intercepts. Circled points indicate animals paired during feeding trials; therefore we averaged mass loss because individual variation in intake could not be controlled in our modelling.

Selection Among Regression Models for Body Mass Change

For nine of the 33 trials, we could not classify the feeding situation (paired vs. solitary) because conditions changed during the trial (when one animal was removed). This problem did not allow us to estimate individual intake during the feeding trial, so we excluded these cases from the model selection analysis. In comparing models fit to the remaining 24 feeding trials, grass consumption was overwhelmingly the most important factor in determining mass change in captive elk (Table 3.2), consistent with the pattern that is obvious in the raw data (Fig. 3.2). Models that did not include any effect of grass consumption performed the worst. Further, all six models that included the quadratic effect of grass consumption performed better than the 5 models that included only the linear effect. We infer that the proportion of grass in the diet has strong but asymptotic effects on winter mass loss.

The effect of nitrogen was less consistent, indicated as important only if grass consumption was already included, thus the linear and quadratic effects of nitrogen were included in the top 2 models. Intake was identified as important in 2 of the top 3 models, but was not in the best model, and was in the worst models. Metabolic body mass and feeding situation were less important as these variables were not in any models within 2 AIC units of the top model. Browse type was not important in explaining variation in mass dynamics for those animals consuming either conifer or deciduous browse (ANOVA, $F_{1, 15} = 0.382$, $P = 0.546$). None of the top 3 models was improved by adding browse type as a categorical parameter (Table 3.3).

Table 3.2: Model selection results using AIC for 18 combinations of 7 parameters to explain change in body mass in captive adult female elk fed winter diets that varied in the proportion of grass and browse.

Rank	Model design parameters[1]							AIC	L.Ratio	P
	1	2	3	4	5	6	7			
1	grass	$grass^2$	nitrogen	$nitrogen^2$				-23.5685	28.38326	0.000010
2	grass	$grass^2$	nitrogen	$nitrogen^2$	Intake			-23.2566	30.07134	0.000014
3	grass	$grass^2$	intake					-21.8990	24.71379	0.000018
4	grass	$grass^2$	intake	mass	Situation			-20.9633	27.77810	0.000040
5	grass	$grass^2$	nitrogen	$nitrogen^2$	Intake	mass	situation	-20.8961	31.71086	0.000046
6	grass	$grass^2$						-19.3593	20.17411	0.000042
7	grass	intake						-18.0993	18.91408	0.000078
8	grass	nitrogen	intake	mass	Situation			-17.4766	24.29141	0.000191
9	grass	nitrogen	intake					-16.9290	19.74380	0.000192
10	grass	nitrogen						-13.9586	14.77336	0.000619
11	grass							-13.5567	12.37146	0.000436
12	intake							-7.9583	6.77305	0.009254
13	nitrogen	$nitrogen^2$	intake	mass	Situation			-7.3392	14.15399	0.014660
14	nitrogen	intake						-6.3397	7.15444	0.027953
15	nitrogen	$nitrogen^2$	intake					-6.1785	8.99324	0.029381
16	intake	mass	situation					-5.9502	8.76495	0.032585
17	nitrogen							-3.0060	1.82079	0.177219
18	nitrogen	$nitrogen^2$						-2.5842	3.39902	0.182773

[1] Variables considered in modeling were grass (% grass in a grass/browse diet), nitrogen (% dietary nitrogen), intake rate (kg forage · 100kg body $mass^{-1}$ · d^{-1}), mass (kg body $mass^{0.75}$), and alone (whether the animal was fed alone or as part of a pair).

The best model, which included the linear and quadratic effects of grass consumption and dietary nitrogen, fit the data well (R^2=0.830, deviance 0.163, df = 11). Positive signs on the coefficients for the linear effects and negative signs on the coefficients for the quadratic terms results in a dome shaped surface when projected in 3-dimensional space (Fig. 3.3, coefficients ± SEM; *intercept*, -1.1851 ± 0.4730, F = 6.28, P = 0.021; *grass*, 0.01349 ± 0.0020, F = 45.84, P < 0.001; $grass^2$, -0.0001 ± 0.00002 F = 22.29, P < 0.001; *nitrogen*, 1.3786 ± 1.0583 F = 1.70, P = 0.208; $nitrogen^2$, -0.5581 ± 0.5976, F = 0.872, P = 0.362).

Table 3.3: Comparative performance of the best models selected by AIC when including or excluding browse type (conifer or deciduous) as an explanatory variable and refit to a data set of mass change in captive adult female elk fed a winter diet that including >0% browse.

Rank	Without browse type					With browse type				
	K	df	AIC	R^2	F (P)	K	df	AIC	R^2	F
1	4	12	-11.98	0.853	17.51 (<0.001)	5	11	-11.98	0.878	15.84 (<0.001)
2	5	11	-14.28	0.901	20.06 (<0.001)	6	10	-12.50	0.903	15.54 (<0.001)
3	3	13	-13.65	0.849	24.44 (<0.001)	4	12	-13.18	0.869	19.90 (<0.001)

Predicting the Optimal Balance of Grazing and Browsing

Although general shape of the surface plot suggests benefits for mass dynamics on diets 50-70% grass:50-30% browse across all levels of dietary nitrogen (Fig. 3.3), the complexity of the best model does not directly identify a single optimal balance of grass/browse that would minimize mass loss. Therefore, to better isolate the relationship between diet composition and mass dynamics, we examined a single, cross-sectional 'slice' taken through the regression surface (Fig. 3.3).

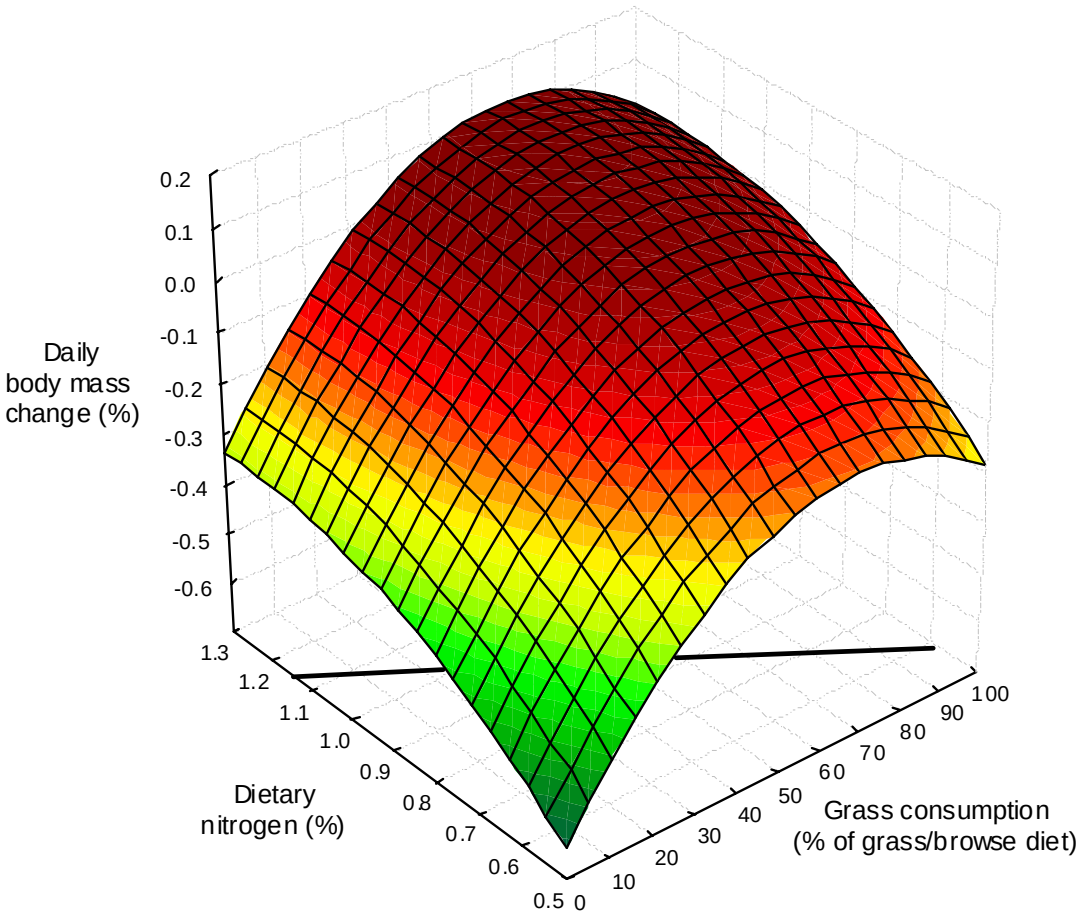


Figure 3.3. The effects of grass consumption and dietary nitrogen on changes in body mass from 24 feeding trials with adult female elk. Model selection using AIC identified this regression as the most parsimonious estimate of change in body mass (% body mass · d-1 = -1.18514 + 0.01349·grass -0.0001·grass2 + 1.3786·nitrogen - 0.5581·nitrogen2). Elk are not predicted to maintain mass if a strict grazer or browser strategy is adopted and if the diet is > 0.8% nitrogen. Generally, increasing nitrogen reduced weight loss in wintering elk. Too little or too much grass in the diet is harmful to mass dynamics. Ideally approximately a half grass/half browse diet that is high in nitrogen (for winter forages) is ideal. The solid line on the lower plane is the location of a cross section through the surface plot where the relationship between dietary nitrogen and grass/browse consumption is an approximation of winter forage conditions in the wild (dietary nitrogen = 1.14 – 0.0054 · % grass in a grass/browse diet).

That is, we examined mass dynamics at varying levels of dietary nitrogen which were determined by the diet composition itself. This approach assumes that dietary nitrogen is linearly dependent on the relative consumption of browse and grass and the nitrogen levels of these forages. Specifically, we examined the response in mass to grass consumption along the function: $nitrogen = 1.14 - 0.0055 \cdot grass$ (Fig. 3.3, solid line). This relationship is developed from mean values (± SE) of nitrogen for graminoid (0.59 ± 0.02%, n = 60) and browse (1.14% ± 0.03 n = 70) forages found on elk winter ranges (Cook & Harris, 1950; Hungerford, 1952; Geis, 1954; Helwig, 1957; Boll, 1958; Johnston & Bezeau, 1962; Cook, 1972; Dietz, 1972; Claar, 1973; Morton, 1976; McArthur, 1977; McReynolds, 1977; Short *et al.*, 1977; Hobbs, 1979; Daneke, 1980; Rowland, 1981; Hobbs *et al.*, 1982; Morgantini & Hudson, 1985; Jenkins & Wright, 1987; McCorquodale, 1993; Wambolt *et al.*, 1997; Wambolt, 1998). Using this function to relate nitrogen content to diet composition, and rescaling daily mass loss to reflect mass dynamics over a 120-d winter, the model predicts that a diet of 53% grass, 47% browse (with 0.84% dietary nitrogen) would minimize winter mass loss (Fig. 3.4). This prediction couples the best general linear model of mass dynamics (from the experimental data) with information on the constraint that diet composition places on nitrogen content (from forages on elk winter ranges). Consistent with many observational studies, the model predicts that most diets would lead to winter mass loss, but it also predicts that adult female elk with an optimal diet could maintain their body mass daily mass over a 120-d winter (+0.64% change in body mass in 120 d).

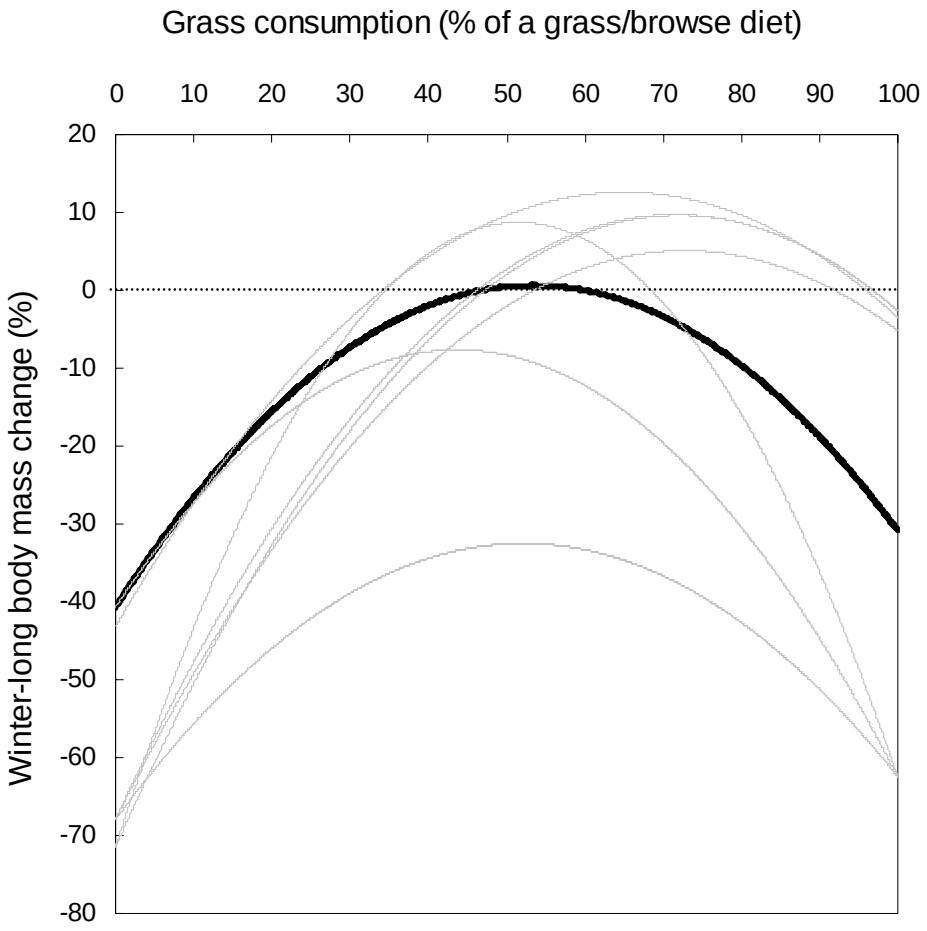


Figure 3.4. Change in body mass from 24 winter feeding trials with adult female elk as a function of grass consumption and at dietary nitrogen levels often found in the wild (solid line, browse nitrogen = 1.14% and grass nitrogen = 0.59%). The relationships shown here are cross sections of the 3-dimensional function where daily change in body mass = $-1.18514 + 0.01349 \cdot \text{grass} - 0.0001 \cdot \text{grass}^2 + 1.3786 \cdot \text{nitrogen} - 0.5581 \cdot \text{nitrogen}^2$. Mass response has been extrapolated over a 120 d winter to reveal cumulative effects. The diet that minimizes loss of body mass is found at the apex of the curve, 53% grass and 47% browse. Systematically varying browse and grass nitrogen to their minimum and maximum values reported in the literature (dashed lines – see text for details) has a greater effect on mass response than on the predicted optimal diet composition.

However, the 95% prediction interval for mass loss with the optimal diet is wide (-8.40%, 9.69%). Given this uncertainty, we tested the sensitivity of predictions about mass loss and diet composition to variation in nitrogen content (Fig. 3.) by alternately varying the absolute and relative values of grass and browse nitrogen to their minimum and maximum values from the literature (grass 0.29% to 1.23%, browse 0.59% to 1.92%). The estimated optimal grass consumption varied little (44% to 72% grass) while the estimate for change in body mass changed greatly (-0.27% to +0.08% mass $\cdot$ d^{-1}) as nitrogen levels for browse and grass varied. Only when browse nitrogen was less than grass nitrogen was the optimal diet > 65% grass.

Discussion

We conclude that the relative amounts of grass and browse in the diet have strong effects on the nutrition of elk, a species commonly classified as an intermediate feeder (Church & Hines, 1978; Hobbs *et al.*, 1983; Perez-Barberia & Gordon, 2001; Clauss *et al.*, 2002). While we did not explore the physiological mechanism behind this result, others have revealed synergistic interactions between grass and browse in the digestive tract of elk (Baker & Hobbs, 1987) and these benefits have been consistently identified in several species of domestic ruminants that also show improved nutrition in response to mixed diets (Rafique *et al.*, 1988; Nunez-Hernandez *et al.*, 1991; Arthun *et al.*, 1992; Bohnert *et al.*, 2002). These studies have also found that some nutritional benefits are unique to mixed diets and have no surrogate in pure grass or pure browse regimens.

Our results provide verification that the balance of grazing and browsing, and the concurrence of these food items within the digestive tract, has consequences for fitness in a wild ruminant. While there may be other synergistic effects that can function over longer intervals between grazing and browsing e.g. browse consumption increasing the circulating urea nitrogen pool for several days which may facilitate grass digestion in subsequent meals, (Rafique *et al.*, 1988), it appears that diets of elk are optimized when mixed over the short-term, i.e., probably less than 3-4 d based on ingesta turnover rates (Baker & Hobbs, 1987). We recognize that fitness is determined by several factors, and natural selection has probably acted on many aspects of foraging behavior and digestive physiology to maximize fitness in ways that do not necessarily minimize winter mass loss (e.g. antipredator responses). Additionally, in wild populations, many factors likely interact to determine mass dynamics (e.g., locomotive costs) or the net benefits of adding grass or browse to the diet (e.g., harvesting costs). That said, variation in diet composition alone explained most of the variance in mass dynamics ($R^2 = 0.716$) corresponding to predictions of 0 to 40% mass loss over a 120-d winter (Fig. 3.4), a biologically significant amount because losses beyond 15% are enough to threaten reproduction or survival (Helwig, 1957; Thorne *et al.*, 1976).

The strong effects of grass and browse on mass dynamics provides evidence for the hypothesis that a specific balance of grass and browse, consumed concurrently over a short time interval, will optimize nutrition for this intermediate feeder (Fig. 3.1). This implies that prolonged bouts of grazing or browsing would be detrimental to mass dynamics in the wild (Fig 3.4.). The general shape of the best model (Fig. 3.3) suggests that a diet of 65% grass, 35% browse is a fairly close approximation of the optimal diet.

This matches the most recent empirical estimate of the winter diet composition of 65.8% grass 29.1% browse, derived from a review of 72 field studies of the diets selected by wild elk (Christianson & Creel, 2007). Our regression model shows that increasing dietary nitrogen has a positive effect on mass dynamics regardless of diet composition and, in concordance with the findings of others (Mould & Robbins, 1981), our model predicts that a diet > 0.8% nitrogen is necessary to maintain mass. However, when differences in the nitrogen concentration between common browse and grass forages from elk winter ranges are considered, the optimum shifts to include more browse. Partly because of the design of the model (i.e., no interaction terms), varying the differences between grass and browse nitrogen never caused the optimum diet estimate to move beyond 44% - 72% grass (56% - 28% browse). We caution that these results likely do not extrapolate beyond winter conditions to times when forage quality is higher and digestive and metabolic constraints are relaxed. Indeed, the negative coefficient on the quadratic term for nitrogen implies that mass dynamics are detrimentally effected when dietary nitrogen exceeds 1.25%, which is unlikely to be true (Mould & Robbins, 1981).

In light of the evidence that an optimum diet exists, temporal variation in the mechanisms that drive the selection of grazing or browsing habitats in intermediate feeders should be an important aspect of nutrition that remains largely unrecognized in the literature. Obviously, mixed diets are not the result of mixed bites; single bites containing both grass and browse are probably uncommon, if not impossible. For elk, browsing and grazing are spatially dependent, correlated with habitat composition, and driven by habitat selection (Christianson & Creel, 2007). Because of this separation in time and space, intermediate feeders must optimally balance their foraging bouts between

browsing and grazing habitats, but how do elk select optimal diets when habitat selection is dependent on many factors not directly related to diet (Creel *et al.*, 2005; Singer & Norland, 1994; Vales & Peek, 1996)? Additionally, at what time scale does the *average* composition of the diet need to be mixed diet to receive the nutritional benefits we discovered? Restated, how many bites of grass or bouts of grazing can the optimal elk consume before moving to browsing areas, for example, without jeopardizing the tenuous mix of grass and browse in the digestive tract, with its benefits for nutrition, and risking the relatively poor payoff of a pure grass diet? This temporal constraint appears unique to intermediate feeders and how it may interact with other constraints is unknown. While others have suggested that grazing and browsing may play alternate roles in the fitness of intermediate feeders, the *frequency* of mixing grazing and browsing and its effects on nutrition may be just as important.

This study also has implications for empirical research. We note that most studies of elk food habits are pooled estimates of grass and browse consumption (across time and individuals) and would detect no difference between elk that consume a 53% grass:47% browse diet every day and elk that graze uninterrupted for 5.3 d and then browse for 4.7. Such groups would vary considerably in their behavior and, more importantly, our data suggest the first group could maintain body mass over winter (Figure 3.4, peak of curve) while the second group would experience significant, and probably lethal, mass loss, even though the long-term diet composition of these two groups would appear to be the same optimal mixture. The importance of variation in the frequency of switching between grazing and browsing for interpreting the nutritional consequences of foraging strategies is highlighted well by a recent review of elk winter food habits (Fig. 3.5). The

winter diet of elk can be highly variable across populations but rarely contains an equal mixture of forages as this study predicts would be optimal. Despite the *average* diet for the species being mixed in apparently optimal proportions (mean ± SD, 65.8 ± 28.1% grass: 29.1 ± 27.2% browse), most elk choose a grazing strategy and in a few places or circumstances, a browsing strategy, but rarely an intermediate strategy (Christianson & Creel, 2007). It is possible that elk are selecting diets optimally, if the sampling interval in these studies was less than the interval over which the diet must be mixed to receive benefits. If elk were foraging as predicted, the distribution of mixed diets in elk populations should be the opposite of that observed (Fig 3.4 & Fig. 3.5).

Specifically, elk graze more than predicted by our mass dynamics model and this overselection for grass appears to be driven by a clear preference for grass and open habitats (Christianson & Creel, 2007). This anomaly has 3 explanations, (1) our model is wrong (2) our model is correct but harvesting costs and other extrinsic factors that we did not consider are far more important for fitness than the singular effects of diet composition on mass, and (3) our model is correct and wild elk do not optimally mix grazing and browsing over the short-term (or necessarily mix at all). It will take a unique approach to describe elk foraging behavior in an appropriate way to test these hypotheses, but considering that pure grass or pure browse diets not only appear lethal (Fig. 3.4), but proved lethal in these studies (Helwig, 1957), it seems likely that some elk populations should be suffering nutritional consequences for grazing biased foraging strategies (Fig. 3.5). The circumstances where consuming a moderate amount of browse would impose larger penalties than not browsing at all are not immediately obvious and seem unlikely.

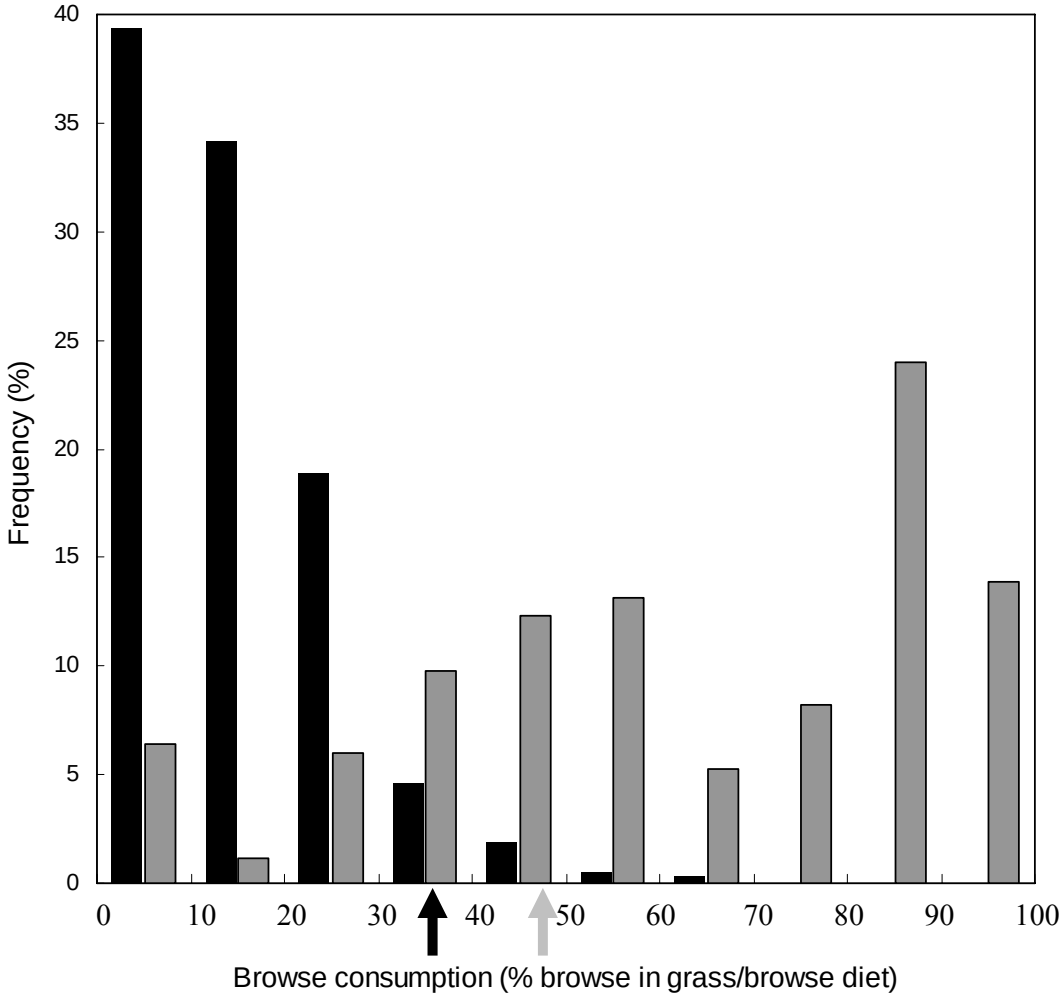


Figure 3.5. The frequency distribution for the percentage of the diet composed of browse for estimates of the winter diet of elk in western North America (adapted from Christianson and Creel 2007). Heavy browse consumption is rare in the majority of winter diet estimates (n = 582) that come from winter ranges with abundant open grasslands or shrub-steppe (black bars). Diet estimates from winter ranges dominated by conifer forests or during severe winter conditions (grey bars) are less common (n = 267) but browse consumption is considerably higher in these populations and can dominate the diet. When diets across all conditions are combined, mixed diets of 30-70% browse (70-30% grass) occurred in only 19% of the 305 published diet estimates. Despite this, the typical diet for this species is often cited as ~65% grass, 35% browse (solid arrow). Foraging responses to environmental conditions appear to cause elk in the wild to consume diets similar to species found at the extremes of the browser-grazer continuum rather than a mixed diet predicted to minimize winter mass loss (grey arrow).

We realize that suboptimal foraging behavior in one of the most widely studied North American ungulates may seem unlikely; yet, while the general ecology and foraging behavior of elk are well described, we know of no studies that have ever confirmed whether the observed pattern of browsing and grazing in wild elk represents the optimal solution to maximizing survival and reproduction. Indeed, there is a considerable body of literature that should warn us against making the assumption that the observed pattern is the optimal one (Gates & Gysel, 1978; Schlaepfer *et al.*, 2002). Maladaptive behavior is especially likely when animals experience recent and dramatic changes in their environment so that current circumstances differ considerably from the evolutionary landscape of their ancestors. In these situations, animals are not likely to possess the optimal solution to novel problems. Certainly, the landscapes that elk now occupy, especially their winter range, fit the criteria of an altered environment (Skovlin *et al.*, 2002). Migratory patterns, predation risk from coursing carnivores, and competition from dominant grazers were likely strong evolutionary forces for the past 500,000 years in North America, but are no longer part of the winter foraging landscape for elk (Kurten & Anderson, 1980; Geist, 1974). Unnatural conditions are further exacerbated as hunting, human disturbance, and grazing by ungulates are prohibited on many elk winter ranges (Wolfe *et al.*, 2002). Could the modification of these important factors be altering the temporal pattern of switching between grazing and browsing in elk today? These hypotheses and the implications of this research can be tested with traditional methods designed to explore the pattern of variation in grazing and browsing. At the very least, exploring the relationship between diet composition and nutrition with empirical data

should provide useful and novel insight into the evolution of foraging strategies of intermediate feeders. Further, refocusing on temporal patterns of diet selection and the environmental factors that drive grazing and browsing should provide useful insight into the evolution of foraging strategies and the ecology of wild herbivores.

CHAPTER 4

FECAL CHLOROPHYLL REVEALS COMPLEX RELATIONSHIPS BETWEEN PRIMARY PRODUCTION AND CONSUMPTION IN A TEMPERATE HERBIVORE

Abstract

In aquatic systems, the concentration of chlorophyll in herbivore feces has been used as a direct measure of the consumption of photosynthetic primary production, but this method has not been applied to terrestrial systems. We measured chlorophyll concentration (greenness) in feces from elk (*Cervus elaphus*) experiencing large fluctuations in primary production in the winter to spring transition over three years. We compared fecal chlorophyll to four indices of grazing herbivore nutrition and habitat quality: fecal nitrogen, grass greenness, grass digestible nitrogen, and landscape-level primary productivity (as measured from satellite imagery by the normalized difference vegetation index or NDVI). We tested whether changes in green biomass consumption were linearly related to changes in primary production (described by NDVI at three spatial scales), a fundamental but untested assumption of herbivore foraging ecology. Temporal trends in fecal chlorophyll were remarkably clear and well described by nonlinear, piecewise regressions ($r^2_a = 0.881 – 0.888$), showing uniformly low concentrations throughout winter followed by an abrupt, rapid increase beginning on different Julian days (88, 91, or 110) each year. Changes in fecal chlorophyll closely matched the temporal trend in chlorophyll and digestible nitrogen concentration of forage grasses collected directly from elk feeding sites. In contrast, fecal chlorophyll deviated, sometimes greatly, from seasonal trends in fecal nitrogen and NDVI. Moreover, NDVI at

foraging sites and consumption of green biomass by elk were not correlated until NDVI reached approximately half its annual maximum, a pattern that became stronger if NDVI was described at broader spatial scales. Combined, these data describe important patterns and constraints on selection for nutritious, green biomass in a temperate herbivore that would be difficult to study without data on fecal chlorophyll. In comparison to the four traditional indices, fecal chlorophyll also produced precise estimates and detected large inter-annual differences in winter length, severity, and rate of spring green-up, as they were experienced by a large, grazing herbivore. Measuring fecal chlorophyll provides a noninvasive, inexpensive, and direct approach to describe an important but relatively unknown aspect of foraging ecology in terrestrial herbivores and may be particularly powerful for studying climate effects in seasonal environments.

Introduction

Primary production, the conversion of carbon dioxide to biomass by autotrophs, creates the energetic foundation of nearly every community on earth. Spatial and temporal variation in primary production is also central to patterns of diversity and evolution in consumers and the dynamics of their populations (Olff et al. 2002, Fritz and Loison 2006). The vital rates of many species fluctuate with the quality and quantity of forage plants and animal life histories are often structured around seasonal patterns of plant growth (McNaughton 1985, Sinclair et al. 2000, Ryan et al. 2007). Differences among consumers are often largely based on foraging strategies that diverge in the type of primary production harvested (e.g., grazers versus browsers) and on the level of selection for photosynthetically active plants (e.g. migratory versus resident populations,

McNaughton and Georgiadis 1986). Understanding the interaction between herbivores and primary productivity has become increasingly important as growing seasons shift, annual patterns are altered, and biodiversity is threatened by global warming and increased atmospheric carbon dioxide (Tucker et al. 1986, Pettorelli et al. 2005, Sparks et al. 2006).

Herbivore ecologists have used several indices to interpret the consequences of foraging decisions for survival and reproduction. A common measure for assessing nutrition in wild herbivores and their food is the concentration of nitrogen in forage plants and feces. Nitrogen is particularly high in the growing and photosynthetically active leaves of plants, which are preferentially consumed by herbivores (Mehaffey et al. 2005). Selection for nitrogen rich plants or patches and the subsequent promotion of these sites from enhanced nutrient cycling are important mechanisms of grazing herbivore ecology (McNaughton et al. 1997, Augustine et al. 2003, Mooring et al. 2005). Interpretation of fecal nitrogen concentrations usually assumes a positive correlation between the consumption and excretion of plant nitrogen: high fecal nitrogen is interpreted as evidence of a high quality diet (Leslie and Starkey 1985, Hernandez and Laundre 2005, Miyashita et al. 2007). However, for herbivores on negative energy budgets, fecal nitrogen includes a mixture of nitrogen from the diet and from catabolism of the herbivore's own proteinaceous tissues (Van Soest 1982, Case 1994). Thus, interpretation of fecal nitrogen as a measure of diet quality is confounded by nitrogen's exogenous and endogenous sources. Moreover, measurements of nitrogen concentration are often expensive and highly variable, limiting inference (Mooring et al. 2005, Murray

et al. 2006). Despite these issues, strong tests of limitations of this index, or suitable alternatives, are few (Hobbs 1987).

A second, more common index used to assess the nutritional value of landscapes, forage plants, and diets can be best described as 'greenness'. For herbivores in seasonal environments, this first approximation to diet quality and selection asserts that that green plant tissue is more nutritious and preferred than dormant tissue (Wilmshurst et al. 1999, Murray and Illius 2000, Macandza et al. 2004, Schrader et al. 2006). A considerable body of heavily cited research confirms that significant variation in herbivore nutrition and foraging behavior can be explained by simple descriptions of the spatial and temporal variation in the greenness of vegetation (McNaughton 1976, Frank and McNaughton 1992, Frank et al. 1998, Sinclair et al. 2000). An increasingly common measure of habitat greenness is the normalized difference vegetation index (NDVI), an indicator to aboveground net primary productivity that is based on remote sensing of photosynthetically active tissue (Tucker et al. 1986, Dawson et al. 2003). Use of NDVI by herbivore ecologists has provided novel insight on foraging behavior, habitat selection and population dynamics (Gustine et al. 2006, van Bommel et al. 2006, Pettorelli et al. 2007, Ryan et al. 2007). However, NDVI data are usually limited to broad temporal or spatial scales and its application to herbivore ecology often assumes that NDVI is linearly related to consumption of high quality, green biomass by herbivores. These assumptions should be tested because animals do not forage randomly, and mechanisms of selective foraging will probably affect the degree to which changes in the plant community at a specific spatiotemporal scale can or cannot be exploited by herbivores (Jarman 1974,

Owen-Smith and Cooper 1987). Surprisingly, the functional relationship between landscape greenness and diet greenness has never been tested for a terrestrial herbivore.

This void is largely due to lack of a method for assessing the greenness of an individual herbivore's diet. The primary photosynthetic molecule in terrestrial plants that reflects green light is chlorophyll (Goodwin 1965) and the concentration of chlorophyll closely tracks the phenology and nutritive value of forages (Billore and Mall 1976, Misra and Misra 1981, Mehaffey et al. 2005). In aquatic ecology, measuring the concentration of chlorophyll in feces has proven a powerful tool for assessing consumption of primary production by herbivores and tracking nutrient flux through ecosystems (Welschmeyer and Lorenzen 1985, Bathmann and Liebezeit 1986, Szymczak-Zyla et al. 2006). Yet, this approach has never been applied to the feces of terrestrial herbivores. Long ago, Reid et al. (1950, 1952) proposed a broad measurement of photosynthetic pigments (that he labeled 'chromagen') in the feces of domestic ruminants and several others attempted to refine the technique (Smart 1953, Deijis and Bosman 1955, Greenhalgh and Corbett 1960, Troelsen 1961). However, the purpose of this technique was to identify a measure of the indigestible proportion of forage crops for captive animals, and the technique has largely been replaced by other indices (Holloway et al. 1981, Van Soest 1982 but see Grant 1971, Boudewijn 1984, Matsui et al. 1989, Lowry and Schlink 1995). Furthermore, the method was never applied to wild herbivore populations, nor considered an index of diet quality. Aquatic ecologists and plant biologists have independently developed powerful techniques for measuring photosynthetic pigments in solution (Arnon 1949, Parsons and Strickland 1963) and these methods appear more appropriate for measuring the greenness of the diet in herbivore feces because they focus on

measuring chlorophyll *a* while those used in Reid's 'chromagen ratio' are more sensitive to concentrations of accessory pigments (carotenoids and xanthophylls, Smart 1953). Adapting the techniques developed for plants and plankton, we should be able to ascertain the chlorophyll concentration of feces from terrestrial herbivores, which, as a measure of diet greenness, should provide novel insight into foraging ecology and nutrition.

In this paper, we present several unique but closely related analyses. Collectively, these analyses constitute a test of the relationship between spatiotemporal variation in primary production by plants and spatiotemporal variation in the diet quality of an ungulate feeding on those plants. (1) We review the literature on the measurement of chlorophyll in biological preparations and present a technique for estimating chlorophyll in terrestrial herbivore feces and forages using spectrophotometry. (2) We demonstrate the use of this technique in a wild population of elk (*Cervus elaphus*) that experienced large temporal and spatial variation in primary production. (3) We use model selection to determine what type of regression on time (linear, quadratic, or piecewise) best explains the temporal trend in fecal chlorophyll and compare this with the temporal trends in four traditional indices of herbivore nutrition and habitat quality: fecal nitrogen, forage nitrogen, forage chlorophyll, and NDVI. (4) We use model selection to determine what type of regression best explains the functional response between fecal chlorophyll and NDVI at the time of fecal collection and we examine how the scale at which NDVI is described affects this relationship. (5) Finally, we address the behavioral and nutritional implications of differences in temporal patterns among the five indices and examine constraints on selectivity for green biomass in elk as revealed by fecal chlorophyll. Our

methods reveal that primary production and ungulate diets do not change in lock-step, both temporally and spatially. We discuss how this approach can compliment current research but also address fundamental questions in terrestrial herbivore ecology.

Methods

Study Area and Data Collection

The Upper Gallatin elk herd (1100 ± 260 animals, mean ± SD, from aerial counts, Montana Fish Wildlife and Parks, 2003-2005) occupies several areas of wilderness in southwest Montana, USA, including Yellowstone National Park (YNP), the Hyalite – Porcupine – Buffalo Horn Wilderness Study Area, the Lee Metcalf Wilderness, the Gallatin National Forest, and the Gallatin Wildlife Management Area. These elk are one of the few populations that escaped eradication in the western U.S. at the end of the 19th century (Peek et al. 1967). Upper Gallatin elk maintain an annual migration to high altitude (>2400 m ASL) summer ranges in June and low altitude (1975 - 2200 m ASL) winter ranges in November, travelling as far as 50 km (Brazda 1953). Migration begins just after the annual birth pulse near 1 June (Johnson 1951) when snow begins melting and grasses and forbs emerge at higher elevations (Brazda 1953). Elk typically return to the winter range in October or November and survive by cratering through snow to reach dormant grasses or by grazing on open south-facing slopes and ridges (Creel et al. 2005). Despite migration and adaptations to foraging in snow, Gallatin elk typically lose body mass in winter, often to the point of starvation (Greer and Howe 1964). Thus, winter and spring conditions are critical drivers of both population dynamics and the synchrony of elk life history with plant phenology (Houston 1982, Singer 1997, Taper & Gogan 2002).

From December through May, 2004, 2005, and 2006, we collected data on elk foraging, nutrition, and environmental conditions on the Upper Gallatin winter range. Our sampling effort was stratified across four tributaries (~125 km^2) of the Gallatin River that constitute the primary winter range of Gallatin elk. Recent and historic observations reveal that the majority of the elk population consistently distributes itself amongst these four creek drainages in winter (Brazda 1953, Peek and Lovaas 1968, Creel et al. 2005). These sites consist of open grass- and shrub-lands in foothills and valley bottoms (1975 m to 2200 m ASL), with dense conifer forest at higher elevations (2200 m – 2400 m ASL).

Fecal and Forage Samples: We located foraging elk groups every 14 d in each of the four drainages and collected 10 fecal samples (~30 ml) from 10 fresh (<24 hrs) and separate piles of elk pellets and stored them at -20°C. We did not pool samples. We sampled feces from similar locations within each of the four drainages throughout the study, but the exact location of a fecal collection was determined by the distribution of elk at the time of sampling. The geographic location of each fecal collection point was recorded with a handheld GPS unit and the habitat type was recorded as either open or forested. Fecal samples from sympatric carnivores such as wolf (*Canis lupus*), coyote (*C. latrans*), black bear (*Ursus americanus*), and grizzly bear (*U. arctos*) were collected opportunistically, for comparison with elk fecal samples as part of the validation of fecal chlorophyll assays (see Results).

As with most elk populations (Christianson and Creel 2007, Cook 2002), grazing in open habitats is the primary foraging strategy used in the Upper Gallatin (Constan

1967, Creel et al. 2005). To assess the nutritional quality of forage we collected forage samples (20.12 g ± 12.56 SD) from elk grazing sites. We collected forage samples less often than feces (see Results) and most intensively in 2005. Forage samples were not total biomass clippings, but hand-pluckings that simulated elk foraging where each 'grab' mimicked one bite (Wallis De Vries and Daleboudt 1994). Forage samples were collected adjacent to fresh elk 'craters' in snow and at sites where we directly observed foraging elk after snowmelt. Hand separation of forage samples confirmed that grass composed the bulk of the harvested vegetation (93.8% ± 13.7 SD) and we refer to forage samples as grass, for simplicity. Grass samples were stored at -20°C, dried for 48 hrs at 55°C, weighed to the nearest 0.01 g, and then ground in an electric mill over a 1 mm screen.

Sample Extraction and Nutritional Analysis: We extracted pigments from fecal and grass samples using a process similar to that used for plant tissue (Lichtenthaler and Wellburn 1983) but originally designed for extraction of fecal hormones, which are structurally similar (Creel et al. 2007). A 2.0 g subsample of feces or grass was dried in a rotary evaporator for 12 hrs. A 0.2 g portion of dried matter was weighed to the nearest 0.001 g and boiled in 95% ethanol for 15 minutes. The pigmented supernatant was separated by decanting after centrifuging. This extract was then evaporated and reconstituted in 1 ml of 100% methanol and stored at -20°C. Most fecal samples and all grass samples were also measured for nitrogen content and digestibility (plants only) at the Wildlife Habitat Nutrition Laboratory, Washington State University, Pullman, USA. For grasses, we combined these measurements into a single nutritional index, digestible

nitrogen (nitrogen × in-vitro dry matter digestibility), to reflect forage quality, specifically, the availability of nitrogen to a ruminant (Mould and Robbins 1981).

Snow and Temperature: To understand temporal trends and patterns of correlation among variables that describe nutrition, we collected additional data on environmental conditions and primary productivity. Winter severity strongly determines diet selection in elk by constraining grazing (Christianson and Creel 2007) and can also affect the date of emergence and growth rate of spring vegetation (Monson et al. 2005). Therefore, we collated data on snowpack and temperature from our study area. For snowpack, daily snow water equivalents (*SWE*) were averaged from three snowpack telemetry (SNOTEL) towers in the Upper Gallatin operated by the U.S. Natural Resources Conservation Service (mean ± SD elevation, 2630 ± 140m). Average weekly temperatures were taken from a weather station located in a drainage adjacent to one of the four primary elk wintering areas and operated by the U.S. National Oceanic and Atmospheric Administration, at an appropriate elevation (2010 m).

Primary Production: The onset of spring-growth and growth rate of green biomass on the landscape are hypothesized to strongly affect the selection for photosynthetically active tissue by herbivores (Pettorelli et al. 2007). Based on the near-infrared and red portions of the light spectrum, the Normalized Difference Vegetation Index (NDVI) provides a well-established measure of above-ground net primary productivity. The NDVI provides a logical comparison with spectrophotometry of fecal and forage extracts, as both methods rely on light absorption by photosynthetic pigments (see below), albeit at different scales and in different contexts. We acquired NDVI

values for the study area from the Global Land Cover Facility (http://www.landcover.org) at the University of Maryland, College Park, USA. The original reflectance data for NDVI comes from bands 1 and 2 using the Moderate Resolution Imaging Spectrophotometer on the U.S. National Aeronautics and Space Administration's Terra satellite. The NDVI data were processed to provide NDVI values ranging from 0 to 250 at a spatial resolution of 250 m and a temporal resolution of 16 d (Carroll et al. 2005). Thus, for each year, 10 NDVI sampling periods spanned the period of fecal collections from 10 December to 1 May.

When the spatial resolution of an NDVI data set is fine in comparison to the area occupied by the study population, some logically-justified form of averaging or subsampling is usually employed (Pettorelli et al. 2005). Using Idrisi32 GIS (Clark Labs, Worcester, Massachusetts, USA) to combine raster layers of NDVI values and fecal collection points, we selected NDVI values from pixels that contained fecal collection points. As we collected all fecal samples from herds directly observed to be foraging, these NDVI values describe the greenness of foraging sites. Again, another filler sentence with enough words to add length to this paragraph so that the gap is not too large at the bottom of the page. Raw NDVI data are prone to errors (Pettorelli et al. 2005) and we excluded NDVI values that were extreme outliers (>2.5 SD's) or when persistent cloud cover prevented an accurate determination of NDVI (Carroll et al. 2005). Excluded data were rare (39 of 1030 NDVI values) and were replaced with the mean of the temporally preceding and following NDVI estimates for that pixel.

Spectrophotometric Technique for Assay of Chlorophyll in Fecal and Grass Extracts

Impetus and Validation: In collecting fecal samples over the winter-spring transition, we perceived a qualitative difference in the seasonal coloration of feces. Measurement of fecal hormones with a spectrophotometric technique (Creel et al. 2007) also consistently revealed a strong green tint in spring extractions that we suspected to be chlorophyll. This prompted us to inquire whether the level of pigmentation might be used as an index to the consumption of photosynthetic tissue with which we could test hypotheses on seasonal foraging behavior and nutritional responses (Creel et al. 2005). Therefore, we reviewed procedures for the quantification of photosynthetic pigments in biological preparations (Arnon 1949, Goodwin 1965, Parsons and Strickland 1963, Lichtenthaler and Wellburn 1983).

We conducted several tests prior to measuring chlorophyll in fecal and forage pigments because the spectrophotometric properties of fecal extracts have not been described for wild herbivores nor have they been used as an index to diet quality. First, we compared the absorption spectra (the intensity of light absorption across all the wavelengths of the visible light spectrum) of elk feces and forages grasses to the spectra of pure photosynthetic pigments (Sigma Aldrich Co., St. Louis, Missouri, USA) to verify that photosynthetic pigments from plant and fecal samples showed patterns of light absorption consistent with known standards. Second, we confirmed that plant pigments are the primary source of the absorption spectra in animal feces. Several organic molecules shared by animals and plants (porphyrins, notably) absorb light in similar ways. If these molecules are excreted in the feces in quantity, they could confound the

measurement of photosynthetic pigments by spectrophotometry. By describing the absorption spectra of feces from carnivores sympatric with elk, we tested whether passage through a mammalian digestive tract might produce such interference. Third, we confirmed that differences in the absorption spectra of elk fecal extracts matched predictions based on broad seasonal differences in the photosynthetic activity of grasses, the elk's primary forage. Most forage grasses are dormant in winter and do not photosynthesize so the absorption spectra of winter grass extracts should not resemble the absorption spectra of photosynthetic pigments, while spring samples should. Elk feces should show this same seasonal pattern if fecal pigment concentration can be used to reliably describe consumption of green biomass. Post hoc, to assess the applicability of this method to other herbivores we also described the absorption spectra of feces collected haphazardly from 15 mammals in North America and Africa and confirmed whether they showed the expected patterns if photosynthetic pigments were present in the diet.

For this first set of analyses, we performed full spectrum scans on pure pigment standards and on extracts from fecal and plant samples, measuring optical density (OD) every 1 nm from 380nm to 780nm for a 200ul aliquot at 1:31 dilution in 100% methanol. We measured OD using a benchtop 96-well microplate spectrophotometer (MQX200, BioTek Instruments, Winooski, Vermont, USA). We identified the optimal dilution for OD measurements by using a two-fold dilution series of a pooled sample from 20 fecal extracts, from undiluted to 1024x dilution. At a 31-fold dilution, peak OD at all wavelengths was clearly defined but <1.0, the condition under which Beer's law is most

likely to be upheld. For presentation in figures (see Results), we averaged the OD at each wavelength across samples to generate the spectral absorbance curves.

Primary Measurement of Chlorophyll in Fecal and Forage Extracts: Having described the absorption spectra of extracts from feces, forages, and pure pigments, and confirmed seasonal similarities in patterns of light absorption between fecal and forage extracts as described above (see Results), we subsequently measured light absorption for the remaining samples at a single wavelength, 666nm (OD_{666}) in our primary analysis because chlorophyll *a* absorbs light most strongly at 666nm in methanol whereas most other pigments do not (Goodwin 1965, Lichtenthaler and Wellburn 1983). Additionally, a peak of absorption in herbivore fecal extracts near 666nm has been found by others (Smart, Jr. et al. 1953), and we confirmed the presence of this obvious and well-defined peak in elk fecal extracts through the validation procedures described above (see Results).

We assayed the OD_{666} of each sample in duplicate. Intraassay coefficients of variation for fecal samples averaged 1.6% and were always <10.0%. The interassay coefficient of variation on a pooled sample of fecal extracts was 1.0%. We corrected each OD_{666} for turbidity by subtracting the OD of the sample at 750 nm. To correct for variation in the mass of plant or fecal dry matter used in the extraction process, we used the equation, $mass\ corrected\ OD = 0.2 \times [OD \times g\ dry\ matter^{-1}]$ (Greenhalgh and Corbett 1960) where the mass of extracted of dry matter was measured to the nearest 0.001 g. This correction factor was always close to one (i.e. we attempted to extract 0.2 g of dry matter for each sample) and allows for direct comparison of chlorophyll concentration

between samples. Under Beer's law, light absorbance by a pigment in solution increases in direct proportion to concentration. For these reasons, we reported only the OD_{666} of fecal and plant extracts (Grant 1971), rather than an approximation of absolute concentration. This approach provides estimates of the relative concentrations of chlorophyll that are directly comparable to one another and would allow for direct comparison across systems. To compare results from our study with those that estimated absolute quantities of chlorophyll, all OD's presented here, after dilutions and corrections, are what would be expected from a 0.00645 g dry matter/ml methanol extraction of plant or feces. For simplicity, we refer to the degree of light absorption at 666nm in plants and feces as chlorophyll or greenness (Grant 1971).

Data Analysis

Describing Temporal Patterns: We compared several regression models for describing annual trends, separately, for each our 5 indices: fecal chlorophyll, fecal nitrogen, grass chlorophyll, grass nitrogen, or NDVI. As discussed above, each of these variables should be strongly affected by plant phenology as winter progresses into the growing season. In describing temporal patterns, our primary goals were to identify what type of regression on time (what functional form) fits best for each dependent variable and to compare the insights into elk herbivory and nutrition provided by these various indices. We considered three functional relationships between each of our dependent variables and our independent variable (*Julian day*): linear, linear with a quadratic term (hereafter, quadratic), or a discontinuous, piecewise regression.

Others have described linear trends between date and nitrogen or green biomass (Frank and McNaughton 1992) and this simplest of models should provide a meaningful comparison to the performance of more complex models. The quadratic relationship also seems logical and is implicitly suggested by many descriptions of decelerating changes in plant phenology, fecal nitrogen, and NDVI during the transition from winter to spring (Massey et al. 1994, Pettorelli et al. 2007). Nonlinear, piecewise regression models are suitable if plants or animals respond abruptly to thresholds (Toms and Lesperance 2003). Others have show that climate and weather can produce nonlinearities in ecological systems (Mysterud et al. 2001) and it seems logical to expect that feces from selective herbivores foraging on plants transitioning from dormancy to growth might show nonlinear patterns. Furthermore, exploratory analysis revealed striking patterns strongly suggesting a nonlinear relationship (see Results). Our piecewise regression model takes the form:

$$y = b_0 + b_1 \times x + b_2 \times [x - breakpoint](x \geq breakpoint)$$

Where y is the dependent variable (e.g. fecal chlorophyll), x is the independent variable (e.g., Julian day), and *breakpoint* is the parameter that identifies the threshold value of the independent variable where the relationship between x and y changes. The parenthetic term ($x \geq breakpoint$) is a logical operator that reduces to 0 (below the threshold) or 1 (after the threshold). The rate of increase or decrease in y is described by b_1 for all x's before the breakpoint and by $b_1 + b_2$ for all x's beyond the breakpoint. Thus, in describing temporal patterns, this four parameter model includes an intercept, two linear phases (rates of daily change in the response variable), and a threshold date when the relationship changes.

Describing the Relationship Between NDVI and Fecal Chlorophyll As a final test of the relationship between primary production and green biomass consumption, we also regressed fecal chlorophyll onto NDVI at the time of each fecal collection. With this approach, we tested whether the greenness of the feces directly responded to the greenness of the vegetation in a linear, quadratic, or threshold manner. To clarify, here the piecewise model describes a threshold NDVI value where the linear relationship between fecal chlorophyll and NDVI might change.

Because 1) NDVI is frequently described at diverse scales, often to minimize the effect of pixel-specific measurement errors, and 2) herbivore fecal composition may be best described by broad (rather than fine) spatiotemporal patterns in primary production (Pettorelli et al. 2005), we described NDVI at three spatial scales at the time of each fecal collection. First, we considered the relationship between NDVI and fecal chlorophyll at the finest spatial scale allowed by the original data set, matching fecal chlorophyll with the NDVI for the single pixel ($250m^2$) at the collection point. As we collected all fecal samples from herds directly observed to be foraging, NDVI at fecal collection points estimates the greenness of foraging sites. We then estimated NDVI at two, broader scales by averaging NDVI values across multiple points for each fecal collection. To describe NDVI at an intermediate scale, we averaged NDVI at all collection points within the creek drainage from which a fecal sample was collected. To describe NDVI at the broadest scale, we averaged NDVI across the entire study area. Overall, these procedures provided three descriptions of NDVI (pixel, drainage, study area) from elk foraging sites at the time of each fecal collection.

For each of the three scales of NDVI, we tested which of the three functional forms (linear, quadratic, or piecewise) best described variation in fecal chlorophyll. Thus, this approach identified the functional relationship between primary production and its consumption by elk, as well as the spatial scale at which differences in NDVI correlated best with differences in fecal chlorophyll. We recorded fecal collection points less precisely in 2004, but due to highly consistent elk distributions across winters, fecal collection points were in close proximity across years (see Results). We excluded 2004 fecal samples from the pixel level analysis, but included these data at the two, broader scales by using the 2005 collection points (when conditions were similar, see Results) to select values from the 2004 NDVI dataset for averaging.

Statistical Analysis: We fit all models by least squares estimation using the GLM module of Statistica (Statsoft Inc. Tulsa, Oklahoma, USA). Fecal chlorophyll (OD_{666}), fecal nitrogen (%), grass digestible nitrogen (%), and grass chlorophyll (OD_{666}), and NDVI for Dec-May 2005 were all independently regressed onto Julian day using linear, quadratic, and piecewise model designs. For each of the three spatial scales describing primary production (250 m pixel, drainage, and study area), fecal chlorophyll was also regressed directly onto NDVI at the time of collection using linear, quadratic, and piecewise designs.

For the piecewise regressions, we used the nonlinear estimation module in Statistica with the Levenberg-Marquardt iteration process to estimate parameters. This module iteratively searched for estimates starting at 0 for all parameters except for the breakpoint. With this process, a single slope is fitted if multiple slopes do not

significantly improve the fit. If two slopes are fit, the breakpoint must lie within the range of minimum and maximum values for the independent variable. We used the midpoint of the range of the independent variable as a starting value for the breakpoint in the iterative process. We compared regression models using Akaike's Information Criterion (AIC, Burnham and Anderson 2002). While we used AIC to select models, we also provide descriptions of model performance with adjusted coefficients of determination (r^2_a). Because the independent variable in models describing temporal patterns was always the same (Julian day), we fit all regressions with standardized variables to allow direct comparison of coefficients. Because our modeling was not concerned with the relative contributions of multiple independent variables and instead attempted to describe the most informative functional relationship with a single factor, and because the parameter estimates have a simple biological interpretation and relevance to our primary questions, a combination of model selection and hypothesis testing was used to facilitate discussions of underlying mechanisms and increase confidence in our results (Stephens et al. 2005).

Results

Plant and Fecal Light Absorption

Absorption spectra of elk feces and grass forage extracts were similar to pure solutions of photosynthetic pigments (Fig. 4.1). Absorption spectra of grass extracts, elk feces, and fecal extracts from 13 other North American and African herbivores showed peaks near 415 nm, 470 nm, and 666 nm, while strictly meat-eating carnivore extracts showed no similar peaks (Fig. 4.1, Appendix B). Black and grizzly bears, which are

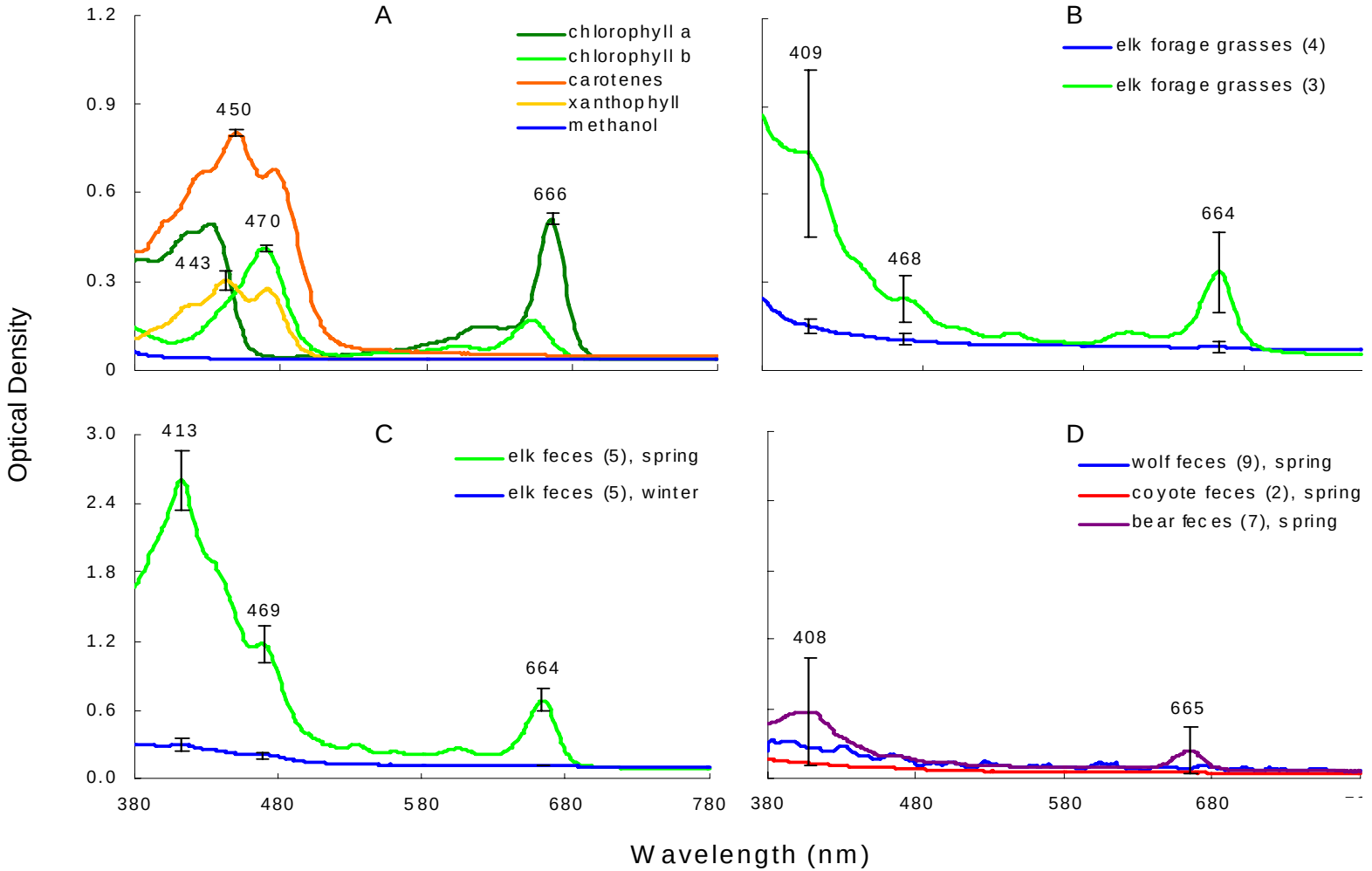


Figure 4.1. Average light absorption from 380 – 780nm (resolution, 1nm) and 95% CI at local maxima for (A) pure preparations of photosynthetic pigments and 100% methanol (used as an extraction solvent), (B) extracts from forage grasses in winter and spring, (C) fecal extracts from elk in winter and spring, and (D) fecal extracts from carnivores sympatric with elk in spring. Light absorption is averaged for each species and parentheses indicate sample size.

omnivores, showed peaks of light absorption at the same wavelengths as herbivores but at lower magnitudes, as expected. Extracts from forage grasses produced higher spectrophotometric peaks in spring than in winter ($t = 11.19$, $P < 0.0001$) as did extracts from elk feces ($t = 13.39$, $P < 0.001$). Given these results, we investigated the temporal pattern of variation in light absorption in all plant and elk fecal extracts using OD_{666}, where light absorption most reflects chlorophyll *a* concentration (Fig. 4.1a). This peak was clear and well defined at the expected wavelength, and chlorophyll *a* concentration is a better index of photosynthetic activity than measures that include the accessory pigment peaks at lower wavelengths (Goodwin 1965).

Temporal Trend in Winter Conditions and Fecal Chlorophyll

Winter conditions varied across years as 2006 was generally more severe than 2004 and 2005 was unusually mild (Fig. 4.2). Less snow accumulated on the ground and snow cover was less variable in 2005 (*SWE* mean ± SD, 26.3 ± 7.9 cm) when compared to 2004 (31.1 ± 12.4 cm) or 2006 (40.8 ± 13.8 cm, $F_{2, 381} = 53.53$, $P < 0.001$). Additionally, a temporary weather station located within one fecal collection site in 2005 showed that snow cover was largely nonexistent after 22 Feb 2005, in an area that is typically snow covered from December-May (Federal Aid Progress Reports, Project 130, Montana Fish Wildlife and Parks, Bozeman, Montana, USA). Average daily temperatures in 2004 and 2005 were 1.90°C and 1.74°C warmer (respectively) than in 2006 (-6.15 ± 5.67°C, $F_{2, 352} = 286.80$, $P < 0.001$).

We measured fecal chlorophyll for 382 elk fecal samples in 2004, 396 samples in 2005, and 244 samples in 2006. When fecal chlorophyll was plotted onto Julian day, the

pattern clearly suggested a threshold response in 2004, 2005, and 2006 (Fig. 4.2) and piecewise regression fit this pattern well (r^2_a = 0.887, 0.881, 0.888, respectively). Linear and quadratic models were ≥249 AIC units worse than piecewise models in all winters, and explained 7 – 54% less variation (Appendix C). Compared to fecal chlorophyll values in spring, chlorophyll remained low and invariant throughout winter (OD_{666} mean ± SE, 0.026 ± 0.0006, n = 786), but early winter fecal chlorophyll differed among years ($F_{2, 783}$ = 19.386, $P < 0.001$). We did not detect any temporal trend in fecal chlorophyll prior to the breakpoint in 2004 or 2005 and a small increase in 2006 (b_1 = 0.0002, P = 0.013, Appendix C). The abrupt onset of 'fecal green-up' as estimated by the breakpoint in the piecewise regression (Fig. 4.2, Appendix C) was highly precise in all winters. The date of fecal green-up (± 95% CI) occurred on March 28 (±2.3 d) in 2004, April 1 (±2.0 d) in 2005, and April 21 (±1.3 d) in 2006. Fecal chlorophyll increased rapidly after these dates, but at significantly different rates each year (Table 4.1, Appendix C). Temporal changes in fecal chlorophyll coincided closely with the spring melt-out (Fig. 4.2). In all winters, fecal chlorophyll rose soon after the mean weekly temperature rose above 0°C and snow ceased accumulating (Fig. 4.2), but it should be restated that snowpack data were recorded at higher elevations than the typical foraging locations of elk at that date. The change in snowpack (from accumulation to loss) at SNOTEL sites coincides with reduced snow cover at lower elevations. In all winters, fecal chlorophyll rose soon after the mean weekly temperature rose above 0°C and snow ceased accumulating (Fig. 4.2), but it should be restated that snowpack data were recorded at higher elevations than the typical foraging locations of elk at that date.

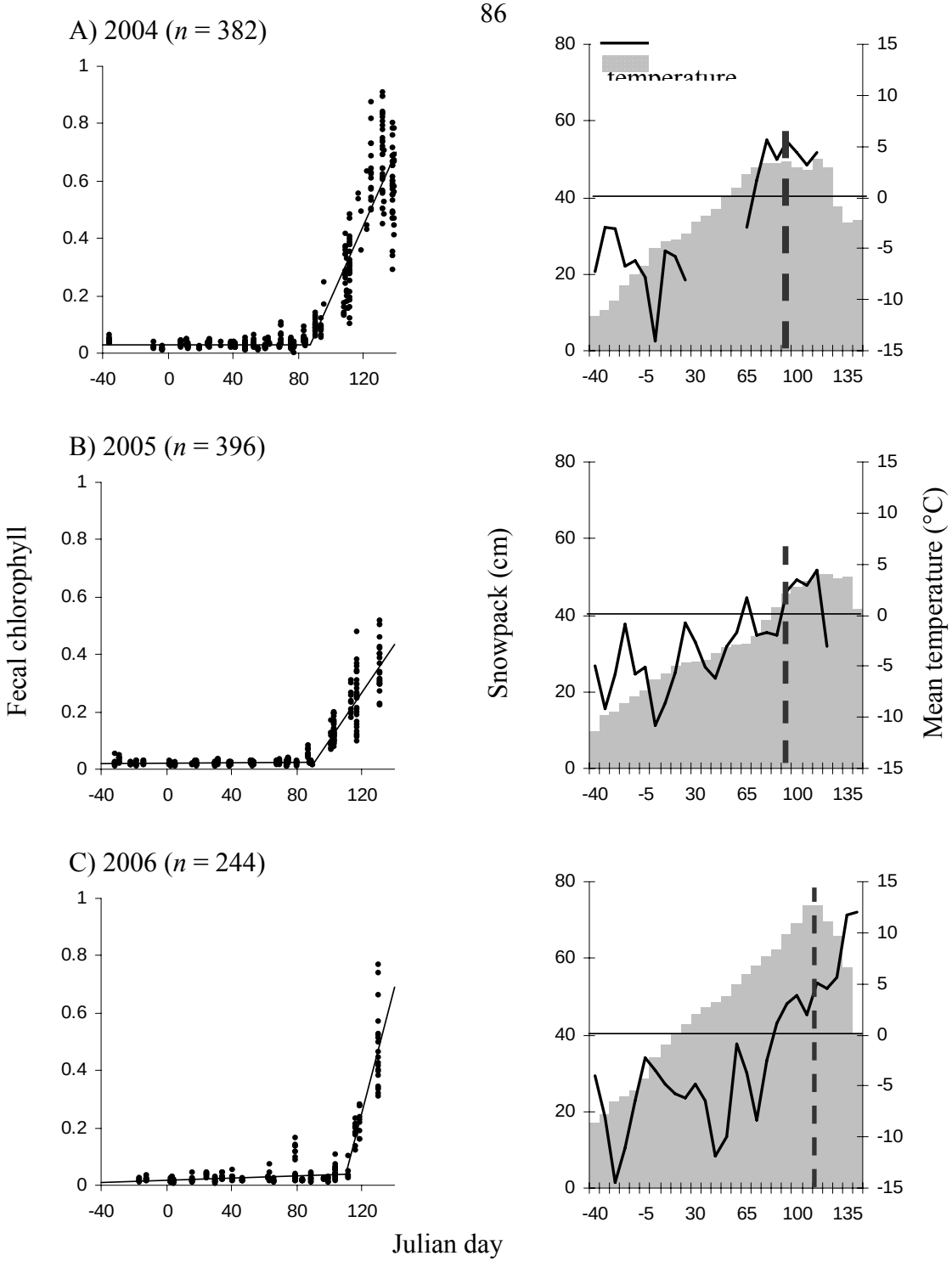


Figure 4.2. Temporal pattern of chlorophyll concentration in elk feces over three winters and correlation with local weather. Piecewise regression (solid lines, left column) fit the data well as fecal greenness (points) remained stable and low in winter but began to rise rapidly after the cessation of snow accumulation (gray bars).

Although our data collection did not consistently extend into late spring when fecal chlorophyll concentrations probably asymptote, the chlorophyll concentration of the final 3 fecal collections in the spring of 2004 (May 19 –May 24, $n = 37$) was slightly less than the 3 preceding collections (May 6 – May 14, $n = 48$) indicating that diet greenness may have stopped increasing near these dates (OD_{666} mean ± SE, 0.683 ± 0.021 and 0.586 ± 0.022, $t_{82} = 3.572$, $P < 0.001$). Furthermore, the late spring fecal samples in all years obtained OD_{666} near the maximum levels we observed for any herbivore (Appendix B). Post hoc, we explored a piecewise regression of all points after the spring green-up (March 28) in 2004 to produce a new relationship modeling the transition from the early-spring rise to late-spring stabilization in fecal chlorophyll ($n = 181$). This new piecewise regression estimated a secondary breakpoint (± SE) 44 days after fecal chlorophyll first began to rise, or May 12 (± 2.4 d, $t_{175} = 56.31$, $P < 0.001$). Consequently, this new regression increases the original estimate for the rate of fecal green-up in 2004 (b_2 ± SE, 0.0156 ± 0.0007, $t_{175} = 21.69$, $P < 0.001$, Table 4.1). This restricted analysis of changes in fecal greenness in late spring is highly preliminary, and is intended only to aid inquiry.

Table 4.1. Standardized effects of Julian day on chlorophyll and nitrogen in elk feces and grass forage and primary production after the estimated breakpoint (date).

Winter	Nonlinear components	Fecal chlorophyll	Fecal nitrogen	Grass chlorophyll	Grass digestible nitrogen	NDVI
2004	$b_1 + b_2$[1]	0.941[2]	0.868			0.809[3]
	Breakpoint	87.7	75.3			66.3
2005	$b_1 + b_2$	0.948	0.797[3]	0.858	0.692	0.720[3]
	Breakpoint	90.8	47.7	87.0	84.4	23.5
2006	$b_1 + b_2$	0.971	0.578[3]			0.835
	Breakpoint	110.4	57.0			81.6

Comparing Fecal Chlorophyll With Temporal Trends in Fecal Nitrogen

Due to their cost (17 \$US · sample^{-1}), our sample sizes for fecal nitrogen were smaller than fecal chlorophyll in 2004 (n = 238, 62% of total) and 2005 (n = 310, 78%). To make comparisons between information provided by fecal chlorophyll and fecal nitrogen, we repeated the above analyses with a data set restricted to samples for which we had nitrogen determinations. Rerunning the piecewise regression of fecal chlorophyll on Julian day using these smaller data sets (one for each year) did not significantly change the parameter estimates of the original regressions (Fig. 4.3a) and AIC values again confirmed that the piecewise regressions of fecal chlorophyll on Julian day had greater information content than linear or quadratic regressions. Compared to fecal chlorophyll, temporal trends in fecal nitrogen were more variable, and the AIC-selected models explained less variance (r^2_a = 0.387 – 0.856). In 2005 and 2006, when sample sizes were largest, linear and piecewise regressions of fecal nitrogen on Julian day were >20 AIC units worse than quadratic regressions, providing no evidence for a threshold response (Appendix D). In 2004, sampling extended later into the growing season which produced high fecal nitrogen estimates with strong leverage (14 of the highest concentrations came from the final 2 collections in May 2004). Consequently, piecewise regression was selected by AIC in 2004 (linear and quadratic regressions were >54 units worse).

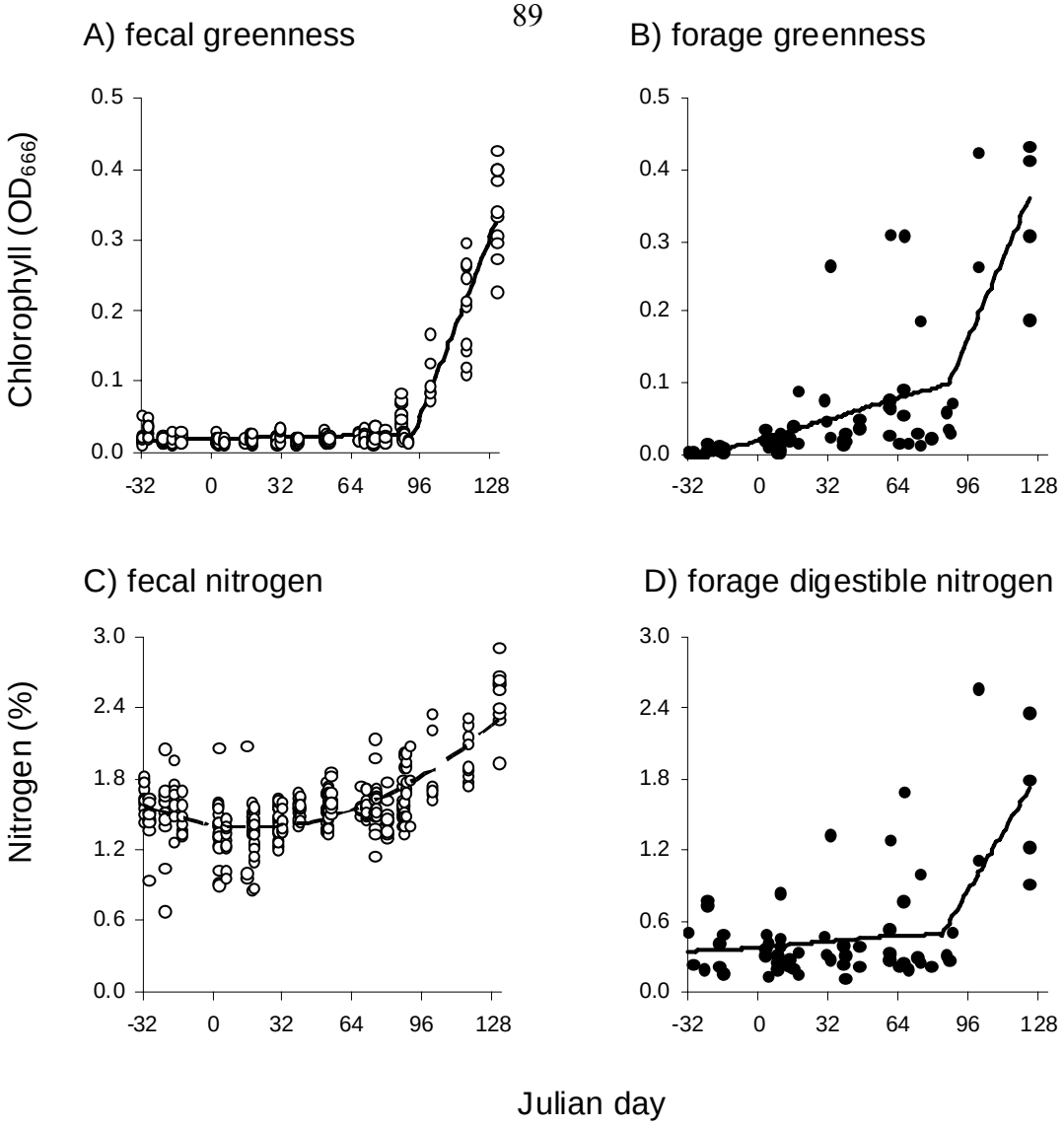


Figure 4.3. Relationship between nitrogen and chlorophyll content and day of winter for elk fecal samples (n = 310) and elk forage grasses (n = 64) in 2005. Note that sample sizes at each fecal collection were approximately equal and fecal chlorophyll values are strongly overlapping in winter in this figure. Elk forage grasses were sampled from foraging areas in a manner that simulated elk foraging. Piecewise regressions (solid lines) explained more variation than quadratic or simple, linear relationships with day of winter except in (C, dashed line) fecal nitrogen where a quadratic regression was selected by AIC. These data highlight the larger variation in nitrogen estimates compared to greenness and the disagreement between (A) fecal chlorophyll and (C) fecal nitrogen while fecal chlorophyll closely matched trends in (B) grass greenness and (D) grass quality.

Both piecewise and quadratic models describe decreasing fecal nitrogen in early winter followed by a slow increase from mid-winter into spring (Appendix D, Fig. 4.3). When we dichotomized the data from each year into winter and spring samples using the breakpoint in fecal chlorophyll, the linear correlation between fecal nitrogen and fecal chlorophyll was positive but weak in winter (coefficient ± SE, 5.43 ± 0.51, $r^2_a = 0.144$, $F_{1,678} = 115.26$, $P < 0.001$) and positive but strong in spring (1.95 ± 0.14, $r^2_a = 0.626$, $F_{1, 111} = 188.56$, $P < 0.001$). However, in winter samples, an ANOVA suggested that fecal nitrogen varied in a manner opposite that of fecal chlorophyll in all three years at three of four sites (Fig. 4.4, fecal nitrogen: $F_{1, 655} = 3.99$, $P < 0.001$, fecal chlorophyll: $F_{1, 655} = 6.72$, $P < 0.0001$). That is, before the spring green-up, elk in drainages with higher fecal chlorophyll generally had lower fecal nitrogen (Fig. 4.4).

Comparing Fecal Chlorophyll With Trends In Grass Nitrogen, Chlorophyll, and Bite Size

At elk foraging sites, grass nitrogen (mean ± SE, 1.12 ± 0.07%, $n = 109$), digestibility, (38.37 ± 1.24%), and chlorophyll content (0.047 ± 0.009 OD_{666}) were low throughout the study period. Although forage sampling in 2004 (n = 17) and 2006 (n = 22) was limited and did not extend past day 95 and 96, respectively, pooling these winter collections across years showed no change in digestible nitrogen ($r^2_a = -0.012$, $F_{1, 38} = 0.534$, $P = 0.469$) and a weak increase in chlorophyll content throughout the winter ($r^2_a = 0.008$, $F_{1, 37} = 4.289$, $P = 0.045$). This weak increase in chlorophyll over winter occurred only in 2004 and was driven by a single grass sample in late winter with high leverage (day 92). After removing this single point, there was no detectable change in grass chlorophyll content ($r^2_a = -0.001$, $F_{1, 36} = 0.974$, $P = 0.330$), similar to fecal chlorophyll.

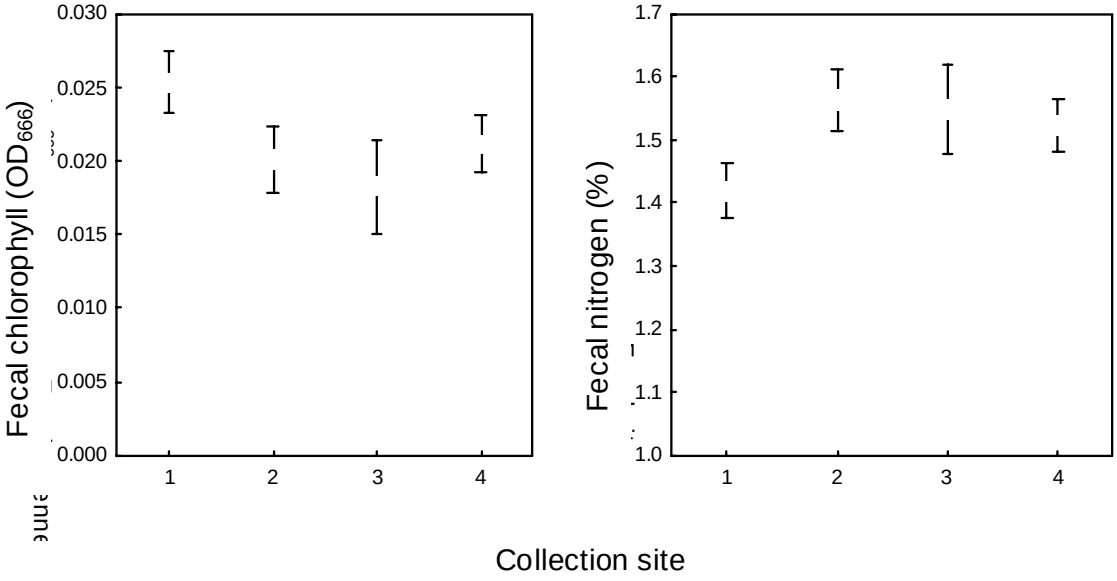


Figure 4.4. Fecal greenness (circles) and fecal nitrogen (squares) from elk samples collected at four sites prior to the onset of spring green-up show opposing patterns. Only data from the winter of 2005 is shown but this inverse relationship between fecal chlorophyll and fecal nitrogen in winter was consistent in 9 of 12 site-years. These areas are delineated by tributaries of the Gallatin River and are separated from one another by roads, rivers, and steep forested canyons except for sites 3 & 4 that are directly adjacent. Landcover classification (Wildlife Spatial Analysis Lab, Missoula, Montana) shows that site 1 is dominated by xeric shrublands (*Artemesia spp.*) and stands of Douglas fir (*Pseudotsuga* menziesii) while sites 2, 3, and 4 in the southern end of the study area are dominated by bare soil, low to moderate cover grasslands, and mixed stands of Douglas fir and Lodgpole pine (*Pinus contorta*).

We restricted model selection to 2005 when sample sizes were larger (n = 64) and extended into the growing season. Temporal patterns of chlorophyll and nitrogen in forage grasses in 2005 were best described by piecewise regressions (r^2_a = 0.576 and 0.401, respectively, Appendix E) and linear and quadratic regressions were >2 AIC units worse. Plant digestible nitrogen and chlorophyll content remained essentially unchanged until day 84.4 and 87.0, respectively, when each began to abruptly rise, in close agreement with the temporal trend in fecal chlorophyll, but with a less-clear relationship

to trends in fecal nitrogen (Table 4.1, Fig. 4.3). Digestible nitrogen and chlorophyll content in grasses were more strongly correlated than they were in feces (coefficient ± SE, 4.21 ± 0.21, $r^2_a = 0.807$, $F_{1, 100} = 424.58$, $P < 0.001$). Chlorophyll content in forage grasses rose more quickly than nitrogen as revealed by standardized regression coefficients (Table 4.1). Temporal trends in fecal chlorophyll suggest little effect on diet from rare patches of green biomass in winter (Fig 4.3a vs. 4.3b, outliers).

Chlorophyll content and estimated bite mass ($g \cdot bite^{-1}$) from hand-grabs at elk forage sites showed a striking pattern as bite size was highly variable in dormant grasses (low chlorophyll) and decreased as chlorophyll content increased (Fig. 4.5, coefficient ± SE, -0.064 ± 0.028, $r^2_a = 0.041$, $F_{1, 100} = 5.19$, $P = 0.025$). This suggested a temporal constraint in bite size, dependent on plant phenology. Because the onset of the growing season as estimated by NDVI varied each year (see below and Table 4.1), we regressed bite size onto day of collection relative to the onset of spring growth for each year (rather than Julian day), to confirm a decrease in bite mass as winter progressed into the growing season (coefficient ± SE, -0.285 ± 0.093, $r^2_a = 0.072$, $F_{1, 106} = 9.35$, $P = 0.003$). This constraint on selection for new growth prevented elk from efficiently harvesting green biomass in quantity in early spring (Fig. 4.5).

Comparing Fecal Chlorophyll with Temporal Trends in Primary Productivity

Annual range for NDVI at fecal collection sites was 32-230 (on an indexed scale of 0-250), while the range in NDVI over just the winter-spring period spanning fecal collections varied from 42-181 (72% of the annual range and 79% of the annual maximum). Thus, spatiotemporal trends in primary productivity held considerable

variation that might explain patterns in elk fecal chlorophyll. Temporal trends in NDVI at fecal collection points generally tracked patterns in fecal chlorophyll concentration (Fig. 4.6). Piecewise regressions of NDVI on Julian day usually performed better than quadratic and linear models (Appendix F); however, in 2004 and 2005, quadratic models were within 2 AIC units of the nonlinear, piecewise regression (Appendix F). Both piecewise and quadratic relationships fit well across all winters ($r^2_a = 0.535 – 0.730$). In the piecewise models, NDVI values were low and relatively uniform until the estimated breakpoint date in all three winters (slope b_1 did not detectably differ from 0, $P > 0.2$) mirroring the pattern seen in fecal chlorophyll (Appendices C, F). However, the estimated onset of green-up from NDVI values was 21.4, 67.3, and 28.8 days earlier than the abrupt rise in fecal chlorophyll in 2004, 2005, and 2006, respectively. Standardized values also show that NDVI increased more slowly than did fecal greenness in all years

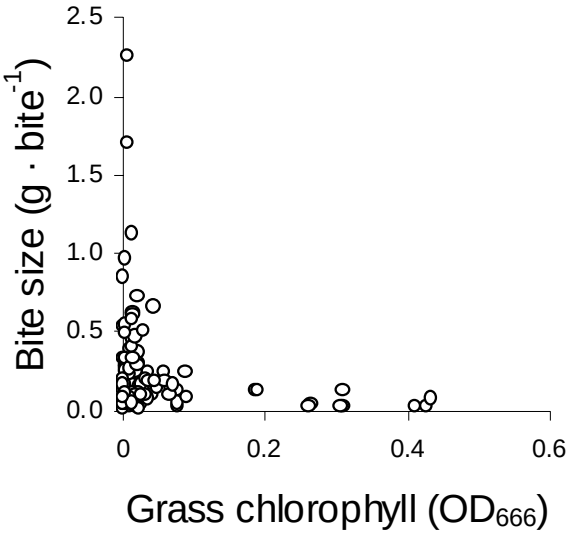


Figure 4.5. Bite size and chlorophyll content for grasses (n = 100) collected from elk foraging sites in winter and spring 2004-2006.

However, the rate of green-up was slowest in 2005 and fastest in 2006, similar to the pattern in elk fecal chlorophyll (Table 4.1). These findings are critical in assessing the ability of elk to benefit from measurable changes in NDVI from satellite data. NDVI data confirmed that the winter of 2005 was exceptionally mild with an early but slowly progressing spring. While the growing season arrived earlier in 2005 than 2004 (*Julian day* ± SE, 23.5 ± 6.5 and 66.3 ± 2.6, respectively), the rise in fecal chlorophyll did not (90.8 ± 1.0 and 87.7 ± 1.2, respectively); however the late arrival of the growing season in 2006 did correspond with a late rise in fecal chlorophyll (Table 4.1, Appendices C, F). . It would seem that despite the mild winter of 2005, elk fecal chlorophyll may have been limited by latitudinal constraints on forage production, as lack of snow cover did not bring about an earlier rise in fecal chlorophyll.

Relationship Between Primary Productivity and Fecal Chlorophyll

We found a strong, direct relationship between NDVI at fecal collection points and fecal chlorophyll, which became stronger when NDVI was estimated at broader spatial scales (Fig. 4.7, Appendix G). Critically, fecal chlorophyll did not respond to increasing NDVI in a linear or quadratic fashion and piecewise regressions were always 14 – 620 AIC units better regardless of whether NDVI was described at the pixel, drainage, or study area scale (Appendix G). Fecal chlorophyll increased weakly across a broad range of low NDVI values, then increased at a much faster rate once NDVI values reached 103 – 131, approximately half the annual maximum in NDVI (Fig. 4.7, Appendix G).

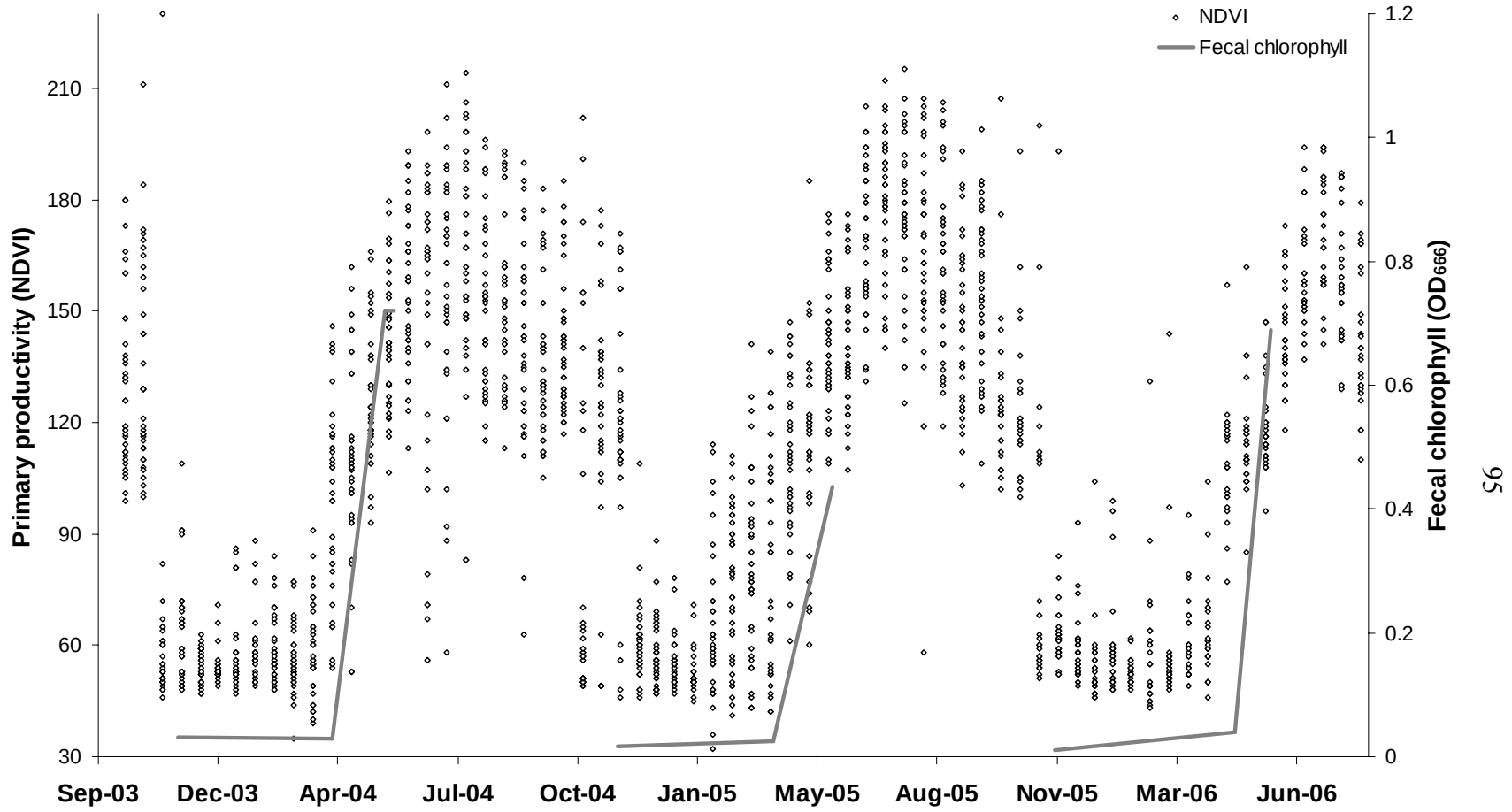


Figure 4.6. Temporal trends in (points) aboveground net primary production, 2003-2006, as estimated by satellite imagery with the normalized difference vegetation index (NDVI) at fecal collection points and (gray lines) fecal chlorophyll from piecewise regressions over the winter-spring transition for 2004, 2005, and 2006. For a visual reference, NDVI values from late May to early November are also shown but were not used in any analysis. Vertical axes are scaled by minimum and maximum observations for each method.

Although all models were significant at $P < 0.001$, NDVI averaged at the broadest spatial scale (entire study area) explained considerably more variation in fecal chlorophyll, in a piecewise manner, than NDVI described at the drainage or pixel level ($r^2_a = 0.777$, 0.567, and 0.461, respectively). Fecal collections from forested areas (where NDVI values might respond differently) were relatively rare ($n = 71$ or 12% of samples with known geographic locations) and removal of these samples from the analysis did not change the model selection results.

Post hoc, we examined whether the unexpected, piecewise relationship between NDVI and fecal chlorophyll was a consequence of sample collection in both winter (when plants are dormant and often snow covered) and spring (when plants are exposed and growing). We dichotomized the data into pre- ($n = 444$) and post- ($n = 678$) growing season using the threshold date for each year estimated by piecewise regression of NDVI on Julian day (Appendix F) and refit piecewise regressions on each data set. Prior to the onset of the growing season (*NDVI* ranged 45-130), piecewise regression could not be fit as there was no linear relationship between fecal chlorophyll and NDVI at any spatial scale ($P = 0.286 – 0.961$). However, for samples collected after the onset of the growing season (*NDVI* ranged 42-181), piecewise regressions were not different from the original relationship we described for NDVI and fecal chlorophyll (Fig. 4.7, Appendix G).

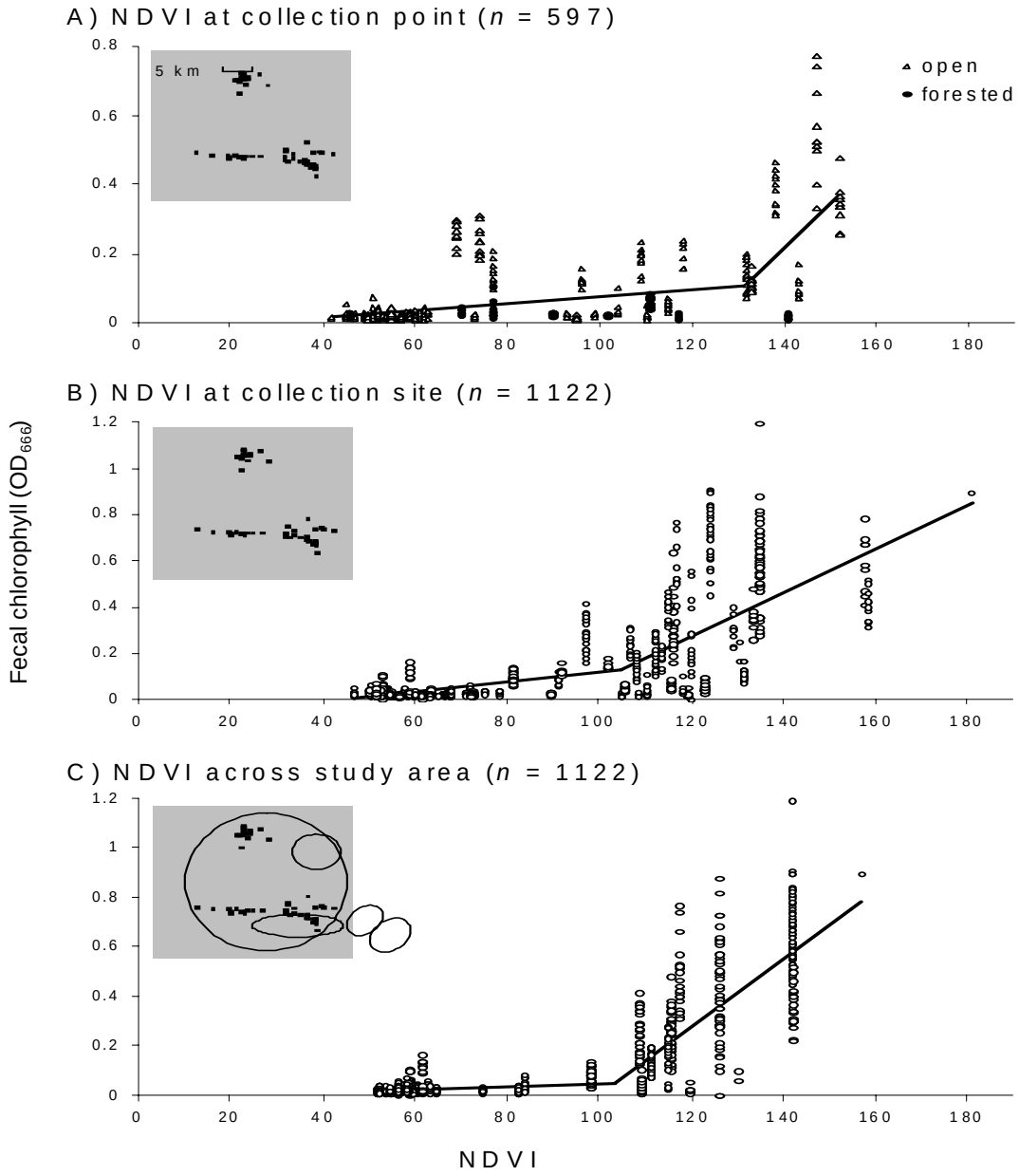


Figure 4.7. The relationship between the chlorophyll content of elk feces collected December-May, 2004-2006 and the greenness of the landscape at the time of fecal collection using the NDVI (A) at the fecal collection point, (B) averaged at all collection points within a drainage, and (C) averaged at all collection points in the entire study area. Note that sample sizes at each fecal collection were approximately equal and low fecal chlorophyll values are strongly overlapping in this figure. The pixel level analysis indicates the prevalence of NDVI estimates from conifer forest dominated pixels, which were rare (12%). Lower sample size at the pixel scale (A) is the result of excluding fecal collections from 2004 when locations were recorded less precisely. Insets shows (A) spatial distribution of pixels that contained fecal collection points and (B and C) spatial scales over which these pixels were grouped for averaging.

Discussion

Our primary hypothesis was that differences in fecal chlorophyll concentrations correlate with differences in chlorophyll concentrations and nutritional quality of consumed plants. We concluded that the optical density of fecal extracts at 666nm (1) could be measured accurately, (2) correlated well with the consumption and availability of green biomass, (3) revealed (by comparison to NDVI) insights about possible limitations on the ability of elk to benefit from the measurable changes in landscape greenness, and (4) may provide new insights on many important aspects of foraging ecology in wild herbivores. By itself, this method revealed simple but fundamental functional relationships between phenology and green biomass consumption that have not been described for terrestrial herbivores. The consistent and strong, nonlinear temporal trend in fecal chlorophyll provided a new way of assessing the timing and rate of change in the spring green-up, *as it is experienced by an animal with specific morphological, behavioral and ecological constraints*. Further, this simple, noninvasive method can be conducted at low cost and great speed (per sample, ~ 0.85 $US and <3.0 min laboratory time), particularly if the less complicated extraction methods used by plant biologists and aquatic ecologists are used (e.g., Lichtenthaler and Wellburn 1983). For questions about the fitness consequences of foraging behavior, habitat quality and conservation or management actions, and comparative studies across species, such a measure will be of substantial use.

Comparisons of fecal chlorophyll with several well-established measures of herbivore nutrition and primary production improved interpretations of seasonal foraging

ecology in this large, temperate herbivore. In particular, temporal patterns in fecal chlorophyll yielded a strikingly different view of elk herbivory than fecal nitrogen (Fig. 4.3), an established nutritional index that can be difficult to interpret in temperate herbivores because of the dual sources of fecal nitrogen (dietary and endogenous). Chlorophyll and nitrogen were more weakly correlated in elk feces ($r^2_a = 0.652$), than in grass from elk foraging sites ($r^2_a = 0.807$) primarily because of a very weak correlation in winter feces ($r^2_a = 0.144$). Moreover, fecal chlorophyll described the temporal trend in forage quality more closely than fecal nitrogen (Fig. 4.3A, B, & D, vs. C). If we had measured only fecal nitrogen, we might logically have concluded that diet quality began to improve in mid-winter. While the mid-winter rise in fecal nitrogen could have a dietary explanation, such a hypothesis must reconcile the lack of a response in fecal chlorophyll (Fig. 4.2) and the strong correlation between digestible nitrogen and chlorophyll content in the elk's primary food. Digestible nitrogen of forage grasses (0.37%) was below the level required to maintain a positive nitrogen balance (Mould and Robbins 1981, Case 1994), and the mid-winter rise in fecal nitrogen is at least partly due to greater catabolism of endogenous nitrogen. This conclusion is supported by opposing patterns between fecal nitrogen and fecal chlorophyll across collection sites that were consistent in 9 of 12 collection site-years (Fig. 4.4); when fecal chlorophyll was low in winter, nitrogen excretion was high. The most parsimonious explanation of these patterns is that nitrogen is sometimes high due to a good diet, and sometimes high due to starvation and catabolism of muscle: by itself, fecal nitrogen produces ambiguous conclusions about herbivore nutrition over the winter-spring period (Hobbs 1987). Fecal chlorophyll provides novel information on diet quality that should not be affected by

winter catabolism of tissue (Boudon et al. 2002), and may sharpen conclusions drawn from fecal nitrogen.

The temporal trend in fecal chlorophyll varied across years and was partially dependent on the rate of primary production as determined by NDVI (Fig. 4.6). Fecal chlorophyll increased slowly in an early spring with slow vegetative growth (2005) and increased quickly after the arrival of a late spring marked by fast growth (2006). However, within this broad pattern there were several nuances that provide potentially important insights on the measurement of relationships between phenology and herbivory. Primary productivity (NDVI) began to rise far earlier each winter than fecal chlorophyll (Table 4.1), and most notably in 2005 when a very early arrival of the growing season had no effect on fecal chlorophyll. Direct measurements of forage greenness at elk grazing sites, collected by simulating elk foraging, suggested that dietary chlorophyll must remain low (Fig 4.3), despite early spring growth, until biomass reaches a threshold (Fig. 4.7). To illustrate, we occasionally detected green biomass at the base of bunchgrass species (e.g., *Deschampsia cespitosa*) at elk foraging sites in winter (outliers, Fig. 4.3b), but our efforts to mimic elk foraging using grab samples often precluded us from selectively harvesting only green tissue without simultaneously sampling the dormant culms and leaves from the previous growing season. Additionally, harvesting new growth at elk foraging sites in early spring was inefficient at first emergence when shoots were short, leading to small but nutritious 'bites' (Fig. 4.5). Fecal chlorophyll confirmed that these same constraints effectively prohibited elk from selectively harvesting green biomass in quantity, as fecal concentrations stayed at winter minimums for a lag period after the onset of the growing season, but then rose abruptly

(Figs. 4.2 & 4.6). These data confirm, in several different ways, that large herbivores like elk cannot exploit sparse green biomass efficiently until densities reach a threshold in spring, an inference that was revealed in our data only by the measurement of fecal chlorophyll.

Further, our comparisons and tests with NDVI data lead us to conclude that the relationship between the density of green biomass on the landscape and its consumption by herbivores may be more complex than is often assumed, for several reasons: (1) Once fecal chlorophyll began to rise, it increased at a faster rate then did NDVI (Table 4.1). (2) Fecal chlorophyll peaked in 2004 (and perhaps in 2006) while NDVI continued to increase (Fig. 4.6). (3) Most directly, the relationship between NDVI and fecal chlorophyll was nonlinear across several spatial scales (Fig. 4.7) and this relationship remained when we only considered samples collected during the growing season. That is, in our most direct test, the abundance of green biomass described at the pixel, drainage, or study area level did not correlate with the consumption of green tissue by elk until NDVI reached approximately half its annual maximum level. Given this evidence, we conclude that it may be dangerous to assume that the abundance of green biomass, as measured by NDVI, is linearly related to its consumption by herbivores. We caution that NDVI may detect large changes or differences in aboveground net primary productivity that are of little consequence to foraging individuals due to constraints on selectivity or preferences for green biomass.

Finally, if winter conditions and plant phenology influence diet, survival, and reproduction in temperate herbivores (Albon 1992, Adams 2003, Stenseth 2003, Mysterud and Ostbye 2006) than quantifying consumption of green biomass may be

important for understanding climate effects on herbivore populations. Pettorelli et al. (2005) recently identified several important seasonal measures to quantify in the study of ecological responses to environmental change. Their focus was on the use of NDVI to describe phenological parameters of primary production that may be important to herbivores. This approach has revealed strong effects on herbivores through variation in winter duration, the rate of spring green-up, and the extent of primary production during the growing season (Loe et al. 2005, Pettorelli et al. 2006, Pettorelli et al. 2007, Mysterud et al. 2007). Here, we uniquely described these parameters *as they were experienced by a large, grazing herbivore* using simple, piecewise regressions of fecal chlorophyll on time. This approach revealed biologically significant inter-annual differences in nearly every parameter (e.g. the onset of fecal greenup varied by as much as 22 d, Appendix C), often in contrast to NDVI. The apparent constraints on selective foraging also suggest that these phenological parameters should vary amongst sympatric herbivore species. Research that combines climate data and fecal chlorophyll, while addressing the role of intrinsic (e.g., morphology) and extrinsic (e.g., predation) factors, may be quite essential for a better understanding of herbivore evolution, foraging behavior, life history strategies, and trophic interactions in the face of environmental change.

CHAPTER 5

RISK EFFECTS IN ELK: SEX-SPECIFC RESPONSES IN GRAZING AND BROWSING DUE TO PREDATION RISK FROM WOLVES

Abstract

Risk effects in ungulates are poorly understood but have recently been implicated as an important driver of elk (*Cervus elaphus*) population dynamics since wolves were reintroduced in 1995-1996 into the Greater Yellowstone Ecosystem (GYE) of Montana, Wyoming, and Idaho, USA. Elk show short-term shifts in habitat use in response to wolf movements which suggest elk may graze less and browse more when wolves are near. This hypothesis has not been tested, but could have important consequences for elk nutrition and for cascading effects of wolves on plant communities. We recorded the daily presence or absence of wolves on three sites occupied by the Upper Gallatin elk population in the northwest corner of the GYE. We estimated the proportion of grasses, conifers, evergreen shrubs and woody stems in 980 elk fecal samples collected from those three sites from December to May in 2004, 2005, and 2006. The winter of 2005 was extremely mild compared to historical records and this anomalous year provided the opportunity to investigate how elk foraging and elk-wolf interactions might change if climate continues to change and winter snowpack continues to decline in western North America. There was considerable variation in the proportion of grazing and browsing. Snow conditions, wolf presence, and elk gender all showed strong but variable effects on the proportion of grass, conifers, evergreen shrubs, or woody stems consumed. Snow accumulation consistently favored browsing and diets during the mild winter of 2005

were dominated by grass, very similar to the spring diet in all years. In normal winters, adult males grazed less than adult females except when wolves were near because adult females decreased grazing in response to wolves. Adult males decreased browsing on conifers by half while adult females doubled conifer browsing on days when wolves were near. Overall, the sexes had different diets (paralleling spatial segregation by sex) when wolves were absent, but showed strong overlap when wolves were present. There were significant interactions between sex, predation risk and snow conditions in their effects on the diet. Adult females responded to wolves strongly in winters of normal severity, yet adult males responded most strongly in the mild winter of 2005 and in spring. Declining winter snowpack in temperate regions are likely to alter the foraging of large herbivores and the interrelationships between plants, herbivores and their predators. Diet shifts due to wolves may be causing trophic cascades that have gone unrecognized and probably carry nutritional consequences for elk that could explain the decreased reproduction since wolf reintroduction.

Introduction

The impact of predators on prey populations has been extensively studied in a wide range of taxa (Begon *et al.*, 1996). When top-down limitation is thought to be important, predators are commonly assumed to limit prey through direct predation (Hanski *et al.*, 2001; Connolly & Roughgarden, 1999; Sinclair *et al.*, 1998). However, a growing body of work shows that predators can also regulate prey through risk effects, or reductions in prey survival, growth, and reproduction due to the costs of behavioral or physiological responses to avoid predation (Werner & Peacor, 2003; Lima, 1998). Risk

effects are often manifested through changes in foraging time or behavior (Lima & Dill, 1990; Schmitz *et al.*, 1997), but relatively few studies have directly tested the consequent effects on the diet of the prey (but see (Beckerman *et al.*, 1997; Eklov & Svanback, 2006; Hodges & Sinclair, 2003). Changes in foraging behavior can also affect lower trophic levels (e.g. primary producers) as prey alter intake rates or diet selection patterns (Schmitz *et al.*, 2004). However, it is unclear if such changes in foraging behavior due to antipredator responses can be generalized across systems (Shurin *et al.*, 2002; Schmitz *et al.*, 2000). Because much of our knowledge of risk effects comes from experiments, often with small animals, it is especially unclear what role risk effects may play in populations of large terrestrial vertebrates and the potential for trait-mediated trophic cascades in natural systems.

The recent reintroduction and conservation of wolves (*Canis lupus*) that primarily prey on elk (*Cervus elaphus*) in the Greater Yellowstone Ecosystem (GYE) of Montana, Wyoming, and Idaho, USA, has generated new opportunities to examine how large carnivores interact with large herbivores in a natural system (Smith *et al.*, 2003). Risk effects might be quite strong in determining elk population dynamics in the GYE because wolf predation risk in late winter is correlated with fecal progesterone levels and subsequent calf recruitment across several elk populations (Creel *et al.*, 2007a). In the Upper Gallatin elk herd, in the northwestern corner of the GYE, the decline in elk population size and calf recruitment following wolf recolonization cannot be directly accounted for by measured rates of wolf predation (Creel and Christianson 2008). It is not known if these risk effects are driven by nutritional consequences of antipredator responses to wolves or some other mechanism (e.g. chronic elevation of glucocorticoid

stress hormones). Wolves alter elk movement patterns (Fortin *et al.*, 2005), grouping behavior (Creel & Winnie, 2005), vigilance levels (Liley & Creel, 2007; Winnie & Creel, 2007), and sensitivity to environmental conditions (Winnie *et al.*, 2006) and these factors seem likely to alter elk energetics or foraging behavior. Most directly, Creel et al. (2005) found that Upper Gallatin elk alter their use of open and forested habitats in response to fine-scale spatial and temporal variation in wolf movement and other studies consistently show that elk habitat use is a strong driver of elk diet selection (see (Christianson & Creel, 2007) for a recent review). Further, predation risk from human hunting has been directly shown to increase the proportion of the diet that is browsed (Christianson & Creel, 2007; Morgantini & Hudson, 1985). Combined, these results suggest the testable hypothesis that elk may graze less and browse more in response to wolves. This hypothesis has not been directly tested, and we know of no studies that have identified the nutritional or physiological mechanism when risk effects have been identified as a driver of population dynamics in a wild vertebrate.

In addition to direct predation and risk effects, the severity of winter is a important component of temperate herbivore ecology, affecting diet selection, survival, and reproduction (Schaller & Ren, 1988; Singer *et al.*, 1997; Mysterud & Ostbye, 2006; Christianson & Creel, 2007). Snow depth and density can strongly constrain foraging by burying grasses (Fancy & White, 1985; Schaefer & Messier, 1996), while cold temperatures and locomotion through snow are energetically costly (Fancy & White, 1987). However, warming temperatures and declining snowpack in recent decades have led to increasingly mild winters in western North America (Mote *et al.*, 2005; Barnett *et al.*, 2005). In fact, the recent winter of 2005 was arguably the mildest in nearly 100 years

of records for the Rocky Mountains (Saunders & Maxwell, 2005). Historical snowpack on our study diet and other elk wintering area in the GYE confirm these same patterns (Wilmers & Getz, 2005). The winter of 2005 was one of the lowest years for April 1 snowpack in 47 years of record keeping in the Upper Gallatin, an anomaly related to the long-term trend towards milder winters with less snow (Fig. 5.1). During the mild winter of 2005, conditions at lower elevations (~ 2000 m ASL) used by elk for foraging were notable for a lack of snow cover by February 10, at least 50 days earlier than long term averages (Christianson 2008; Wildlife Survey Reports, Montana Fish Wildlife and Parks, Bozeman, Montana, USA). While our primary interest was to describe elk winter and spring foraging in the face of predation risk from wolves, the anomalous winter of 2005 also provided the opportunity to examine how this predator-prey interaction may change as winter snowpack continues to decline.

Study Area and Methods

The Upper Gallatin elk herd is an annually migratory population (mean ± SD, 1100 ± 260 animals from aerial counts, 2003-2005 Montana Fish Wildlife and Parks, Helena, Montana, USA) that moves between alpine and subalpine (>2400 m ASL) summer habitats in the Gallatin and Madison mountains of Montana and Wyoming to lower elevation (1975-2200 m ASL) foothills and valleys along the upper Gallatin river of Montana, in winter. We conducted this study from December to May when elk congregated on the winter range. The elk winter range is composed of large expanses of open sagebrush-steppe (*Artemesia* spp.) and grasslands (mostly *Agropyron* and *Festuca* spp.) with steeper slopes and higher elevations dominated by mature conifer forest (*Pinus*

contorta, Picea engelmanii, Abies lasiocarpa, and *Psuedostuga menziesii*). Population monitoring since 1928 confirms a consistent pattern of elk distribution across the Upper Gallatin winter range. Most the elk population divides itself across the four most open drainages of the Gallatin river (Brazda, 1953; Winnie & Creel, 2007; Creel *et al.*, 2005; Peek *et al.*, 1967; Peek & Lovaas, 1968). These four drainages form three distinct sites (two smaller drainages are directly adjacent) that are separated from one another by rugged and densely forested terrain and the Gallatin River and highway 191, which run south to north through the middle of the study area (see (Winnie & Creel, 2007; Creel *et al.*, 2005) for additional descriptions of the study area). Recent and historical data confirm similar densities and little movement by elk between sites in winter (Brazda, 1953; Peek & Lovaas, 1968; Creel *et al.*, 2005).

Data Collection

Elk Diet Selection: We stratified data collection effort across the three sites from December through May in each of three winters: 2004, 2005, and 2006. We collected 3–10 fresh (<24 hrs) fecal samples (~ 30 ml) from separate piles of elk fecal pellets in each of the three sites approximately every 14 days. We used direct observation of elk and snow-tracking to maximize the probability that each collection occurred within a single group of elk and each sample came from a single individual (mean of 8.8 samples/collection). We adapted a microhistological technique to prepare microscope slides of each fecal sample to determine its botanical composition using genus- or species-specific epidermal features of plants (Sparks & Malechek, 1968). After blending in a household blender for 6 min and soaking in 95% ethanol for 7 d, we washed and

bleached (3% sodium hypochlorite for 6 min) fecal and rumen samples over a #200 sieve. We then soaked a 3-ml subsample in lactophenol blue for 7 d before washing it over the sieve. We spread a ~1-mm^3 subsample over a 25-mm^2 area on a 25-mm × 75-mm glass slide and protected the mount with a 25-mm^2 glass cover slip.

We used our slide preparation technique on dried (48 hr at 55°C) and ground (over a 1 mm screen) forage plants collected from the study area to make 59 reference slides for use in identifying epidermal plant fragments on slides made from fecal samples. An independent laboratory specializing in microhistological diet analysis (B. Davitt, Wildlife Habitat Nutrition Lab, Washington State University, Pullman, Washington, USA) found 27 plant genera or species (11 graminoids, 7 forbs, and 6 shrubs, and 3 trees) in a pooled, random sample of elk feces. Reference slides included these 27 forage plants and 32 other common plants and plant parts (stems and leaves) found on the Upper Gallatin elk winter range. To increase the accuracy of assignments (but reducing specificity), we categorized reference plants into six vegetative groups based on similarities in epidermal features. These groups were: (1) graminoids, (2) forbs, (3) conifer needles, (4) evergreen shrub leaves (primarily sagebrush, *Artemesia* spp.), (5) willow/aspen stems (*Salix* spp. and *Populus tremuloides*), and (6) other woody stems (mostly *Ribes* spp.*, Symphoricarpos* spp.*, Rosa acicularis,* conifer and evergreen shrub stems). Epidermal fragments from four plants were readily identifiable to the generic or species level and could not be easily confused for other plants: Douglas fir needles (*P.* menziesii), silverberry stems (*Eleagnus commutata*), Oregon grape leaves (*Berberis repens*), and phlox leaves (*Phlox* spp.). A preliminary analysis involving 112 fecal

samples found that 99.97% of epidermal fragments were classified to one of these 10 categories (i.e., only 3 of 11,200 epidermal fragments could not be identified).

For microscopic determination of each diet, we identified (at 100X) the first 30 epidermal fragments intersected by an ocular crosshair while scanning random, 25-mm transects on each slide using a mechanical stage. Transects ran parallel to one another and perpendicular to the long-axis of the slide with an interval spacing of 1 mm. To avoid observer bias, all slides were labeled with an identification number and all diets were tabulated on a spreadsheet used only to tally fragments so that the observer was blind with respect to all independent variables that were subsequently paired with each diet in the statistical analyses (see below). Further, all diets were determined by a single observer over a continuous six month period in a haphazardly random, non-sequential order. After each diet was tallied, we added Douglas fir fragments to the total count for conifer fragments and added silverberry and willow/aspen stem fragments to the total count of woody stem fragments. We combined counts of graminoid, forb, Oregon grape, and phlox fragments into a single category to describe grazing – the portion of the diet that is primarily consumed while the head is down, usually in open habitats (Christianson & Creel, 2007). Forbs, Oregon grape, and phlox usually formed a very small portion of the diet that was positively correlated with graminoid consumption (see Results) so combining these plants with grass into a single category had little effect on results. For simplicity, we refer to this category as grazing or grass. These procedures yielded four broad categories to describe the diet of elk: % grazing, % conifers, % evergreen shrubs, and % woody stems. These categories are well suited to detect potential changes in elk diets due to shifts in habitat selection or shifts in micro-site foraging patterns.

Wolf Predation Risk: Wolves recolonized the upper Gallatin in 1996 following a wolf reintroduction program in neighboring Yellowstone National Park, Wyoming, USA in 1995 –1996 (Fritts *et al.*, 1997). From 2004-2006, wolves persisted across the study area (2 – 3 packs, 8 – 17 wolves) and regularly moved between drainages or off the study area, creating temporal and spatial variation in predation risk for elk (see Results). We recorded whether wolves were detected or not each day that any data were collected within a site. Radio-collars were maintained on 0 – 4 wolves throughout the study period as part of population monitoring effort (U.S.Fish and Wildlife Service *et al.*, 2005; Sime *et al.*, 2007). Wolf sightings, radiotelemetry, howls, or fresh (<24 hrs) tracks, kills or scats confirmed if wolves were present within a site on any given day. We scored wolves as absent from a site if they were not detected by any of these means. While we undoubtedly failed to detect wolves on some occasions, this method is conservative in that detection failures would tend to underestimate the effects of wolves on the diet. Previously, this method has detected effects of local wolf presence on elk behavior, grouping, habitat selection and sensitivity to environmental conditions.

In describing effects of predation risk on elk foraging, we recognized that fresh fecal samples contain information on the foraging decisions made by elk over the previous 2-3 days (Jiang & Hudson, 1996). To address the time lag between plant consumption and fecal collection, we also considered whether wolves were detected in a site over the two days preceding the day of fecal collection. That is, we dichotomized elk diets as being consumed with “wolves present” or “wolves absent” depending on whether or not wolves were detected within the site on the day of, the day preceding, or two days

preceding fecal collection. If wolves were never detected in those three days, we considered wolves to be absent (note that wolf locations are strongly autocorrelated at this spatiotemporal scale (Bergman *et al.*, 2006). We excluded samples that were collected when no determination of wolf presence or absence had been made.

Snow: Snow can increase the vulnerability of prey to predators by restricting movement (Huggard, 1993; Bergman *et al.*, 2006). Snow depth and density can also affect diet selection in herbivores by restricting access to herbaceous vegetation, especially in those intermediate feeders that prefer grazing but also browse (Jenkins & Wright, 1987; Adamczewski *et al.*, 1988; Skogland, 1978; Telfer & Kelsall, 1984; Christianson & Creel, 2007). We measured snow depth and compaction by dropping a 3-kg steel ball from 30 cm and recording its penetration and total snow depth to the nearest 1 cm at three fixed points in each site every 14 d (Winnie *et al.*, 2006). We used penetration and depth to scale snow compaction from 0 (soft, powdered snow) to 1 (impermeable crusts) with the formula, $compaction = 1 - (cm\ of\ penetration / cm\ of\ total\ snow\ depth)$. For each site and 14-d period, we joined the minimum snow depth and maximum compaction with the formula, $snow\ index = snow\ depth_{min} + snow\ depth_{min} * compaction_{max}$. We used the minimum snow depth (of the three collected within each site and 14-d period) because elk can forage selectively and will preferentially select areas free of snow due to wind or snow-melt (Houston, 1982) and mean or maximum snow depth could fail to capture this ecological circumstance. For each site, we matched each fecal collection with the snow index measurement nearest in time (Fig. 5.1B).

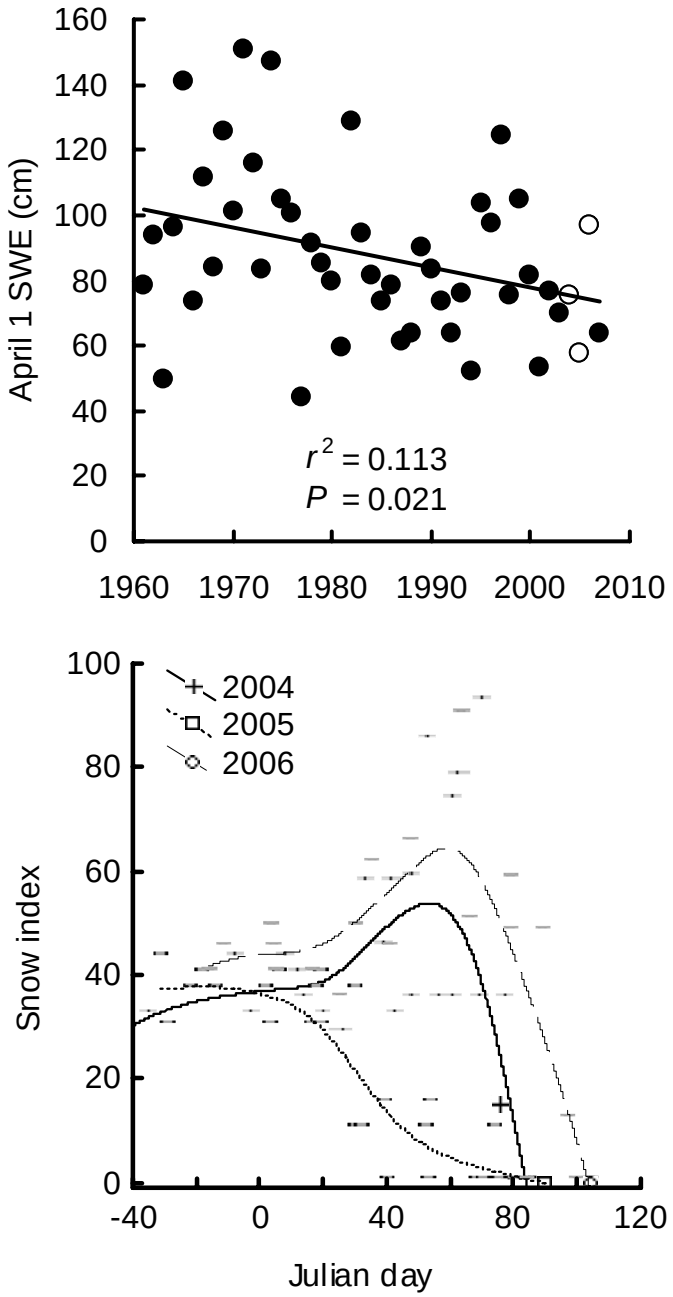


Figure 5.1. (A) April 1 snow water equivalents (SWE) from 1961 to 2007 for the Carrot Basin snowcourse site in the Upper Gallatin, Montana, USA (U.S. Department of Agriculture, Natural Resource Conservation Service, http://www.wcc.nrcs.usda.gov). Snowpack has been steadily decreasing for several decades and (open points) the three years of this study included 2005, one of the mildest winters on record. (B) A more detailed profile of changes in snowpack on the Upper Gallatin elk winter range through winter and spring for the three years of this study.

Sex: Elk are sexually dimorphic, polygynous ungulates, and adults assort into herds with highly biased sex ratios (often single-sex herds) in the Upper Gallatin in winter (Winnie & Creel, 2007; Peek & Lovaas, 1968; Creel & Winnie, 2005; Creel *et al.*, 2005). This sexual segregation was used to classify fecal samples according to sex based on the sex bias of groups or sites and fecal pellet size (Bleich *et al.*, 1997; Vales & Peek, 1996). Frequently (58.57% of all fecal samples), the composition of a group (number of cows, calves, yearling males, and adult males) was directly observed immediately prior to fecal collection. In addition, we walked fixed transects every 14 d in each drainage and used systematic scans of the entire viewshed with binoculars and spotting scopes to locate and classify every elk group. While regularly collecting other data on elk foraging behavior, wolf predation, and environmental conditions throughout the study area, we also scanned for elk, counting and classifying every group detected. If the group or drainage composition was directly observed before fecal collection, we classified samples according to sex based on the proportion of adult males ('bull' if >85% or 'cow/calf' if <15%). If the group or recent drainage compositions were of mixed sex (85% ≥ %male ≥ 15%) at the time of fecal collection, we used fecal pellet size to distinguish between the sex of individuals (Maccracken & Vanballenberghe, 1987; Khan & Goyal, 1993). Sex-specific pellet size was determined from probability density functions of pellet size developed for calves, adult females, and adult males using fecal pellets from carcasses collected opportunistically on the study site (Fig. 5.2: Christianson 2008). Sexual segregation was pronounced, so most samples (88.0%) could be classified using the group or drainage sex bias: of 13104 elk in 1138 groups classified over the three winters, 92% of all elk were in groups that were either >90% or <10% adult male.

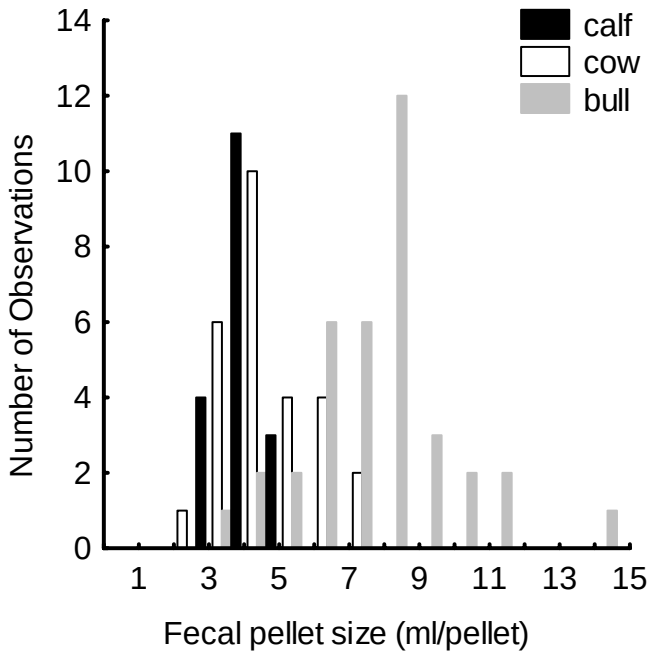


Figure 5.2. Frequency distributions for pellet sizes from elk of known age and sex in the Upper Gallatin Canyon in winter. Pellet size was measured by dividing the gross volume occupied by the number of pellets in a clear, graduated 50ml polypropylene centrifuge tube. The probability density functions developed from these data were used to assign age-class probabilities to fecal samples collected from groups of mixed sex (see Methods). Samples larger than 6.4 ml/pellet (arrow) were classified as 'bull' and smaller than 6.4 ml/pellet as 'cow/calf'.

Data Analysis

Broad inter- and intra-annual variation in environmental conditions can interact with elk behavioral responses (Gude *et al.*, 2006; Vales & Peek, 1996; Singer & Harter, 1996; Singer & Norland, 1994). Further, climate change is predicted to effect herbivores through changes in winter energy demands and forage plant phenology (Mysterud & Ostbye, 2006; Chan *et al.*, 2005; Pettorelli *et al.*, 2005). Because the winter of 2005 was exceptionally mild (Fig. 5.1), we separated samples collected in this 'mild winter' from the 'normal winters' of 2004 and 2006 and conducted a separate analysis on each winter type. With this approach we examined how diets and elk-wolf interactions are affected

by the background level of winter severity which provided insight on how these relationships may change due to increasing atmospheric temperatures and declining winter snowpack (Barnett *et al.*, 2005).

Although our primary focus was to test for dietary responses to wolves in normal winters when the nutritional consequences of constraints on foraging may be most significant, we found that an analysis of spring diets provided an important contrast that facilitated interpretation of elk foraging ecology. Elk life history and population dynamics appear strongly linked to the period when snow cover no longer constrains grazing and new plant growth provides a highly nutritious alternative to the dormant culms and leaves that sustain elk through winter (Taper & Gogan, 2002; Vore & Schmidt, 2001; Singer *et al.*, 1997). However, defining the onset of spring in terms relevant to herbivory has often proven difficult. Fixed Julian dates, such as the vernal equinox, can be arbitrary and do not allow for substantial latitudinal, altitudinal, or annual variation in the rate of snowmelt or the onset and rate of spring growth (Pettorelli *et al.*, 2005). Additionally, regardless of when spring may 'arrive', herbivores vary considerably in their degree of selectivity for new spring growth so that the demarcation between winter and spring nutrition may be quite different across species in similar seasonal environments. To separate spring samples from winter samples we used the concentration of chlorophyll in elk fecal samples as measured by spectrophotometry of fecal extracts (Christianson and Creel *in review*). Specifically, we dichotomized diets based on their collection date by using the day after which fecal chlorophyll concentrations showed persistently elevated levels, indicative of increased intake of photosynthetically active plant tissue. To determine this date, we used the breakpoint

estimated by a piecewise regression of fecal chlorophyll onto Julian day in each winter. Mean chlorophyll concentration between winter and spring samples differed by a factor of 12 so this demarcation date was unambiguous and clearly defined in each year. The date (± 95% CI) of demarcation between winter and spring differed significantly across years: March 28 (±2.3 d) in 2004, April 1 (±2.0 d) in 2005, and April 21 (±1.3 d) in 2006, and unlike traditional descriptions of the onset of spring, this measure described the date at which elk begin to consume green biomass in quantity (Christianson and Creel *in review*). In this manner, we identified the timing of spring green-up as it actually affected elk diets.

A visual assessment of the normalized response variables (% grazing, % conifers, % evergreen shrub, or % woody stems) confirmed nonnormality due to biases in the frequency of diets towards 0% and 100%. Therefore, we transformed the response variables using the logit transformation on adjusted proportions (Fox, 1997). We used analysis of covariance (ANCOVA) within each winter type (normal and mild) to explain variation in each diet component as a factor of wolf presence and elk sex while controlling for the snow index, a continuous covariate. We used analysis of variance (ANOVA) to explain variation in spring diets considering the effect of wolves and elk sex. We standardized all variables before analysis to allow direct comparison of their effect sizes. Because logit transformed means cannot be directly interpreted and confidence intervals from logit transformed data are asymmetric, we report back-transformed 95% CI from ANCOVA's (with snow controlled at its mean) or ANOVA in the text for simplicity and to facilitate comparisons and interpretations of biological significance.

Results

General Patterns in Elk Diet Selection

We determined the diet composition for 980 fecal samples. Within this single population, elk showed a broad scope of variation in their use of grazing and browsing strategies: diet ranges including 0–100% grazing, 0-90% conifers, 1–53% evergreen shrubs, and 0-100% woody stems. As is typical of elk, graminoids formed the bulk of the winter diet (83.3% of samples contained >50% graminoids). The least grazing occurring in normal winters and the most grazing in the extremely mild winter of 2005 and in spring (Table 5.1): this effect is also typical of elk. Forbs (mean ± SD, 4.0 ± 7.1%), Oregon grape (2.1 ± 5.0%), and phlox (0.5 ± 1.6%) always made small contributions to the diet (Table 5.1) and were positively correlated with graminoids in the diet ($r = 0.068$, $P = 0.034$) providing justification for combining these variables into a slightly broader diet component, grazing. In spring, grazing heavily dominated the diet in 2004, 2005, and 2006 (91.4 ± 9.7%, $n = 108$; 96.3 ± 4.0%, $n = 101$; 92.3 ± 16.5%, $n = 44$; respectively) and spring sample sizes were smaller than in winter so we pooled spring samples across years.

Wolf predation risk within sites varied considerably (Fig. 5.3). We determined 401 diets for samples collected at times when wolves had not been detected and 420 diets collected at times when wolves had been detected, providing high power to test for the effects of wolf predation risk on elk diets. We could not confirm wolf presence or absence on the day of, or the two days preceding fecal collection for 159 samples and these samples were excluded from ANCOVA's and ANOVA's.

Table 5.1. Diet composition (% mean ± SD) for major sex-class groups of elk under various seasonal conditions.

Diet item	Normal Winter		Mild Winter		Spring	
	Cow-calf	Bull	Cow-calf	Bull	Cow-calf	Bull
n	313	128	192	85	203	42
Graminoids	65.6 ± 19.0	56.3 ± 20.8	83.8 ± 9.7	76.9 ± 12.5	80.4 ± 14.4	76.8 ± 19.7
Conifer	12.0 ± 12.5	24.6 ± 18.0	3.6 ± 5.1	11.0 ± 9.4	1.6 ± 3.2	5.6 ± 5.9
Evergreen shrubs	9.5 ± 9.3	7.9 ± 9.8	4.7 ± 5.7	4.4 ± 5.0	1.2 ± 2.3	1.8 ± 3.5
Woody browse	8.5 ± 8.6	9.9 ± 11.3	2.7 ± 3.5	3.2 ± 3.8	1.7 ± 2.9	6.3 ± 16.2
Forbs[1]	2.2 ± 4.0	0.8 ± 2.0	2.6 ± 3.6	2.7 ± 3.7	10.2 ± 11.7	4.0 ± 3.9
Oregon grape[1]	1.7 ± 3.4	0.3 ± 1.7	1.9 ± 3.4	0.7 ± 1.8	4.4 ± 7.6	4.8 ± 10.6
Phlox[1]	0.5 ± 1.4	0.2 ± 1.0	0.5 ± 1.5	1.1 ± 2.1	0.6 ± 1.7	0.6 ± 1.7

[1] This diet item was combined with graminoids prior to the main analyses.

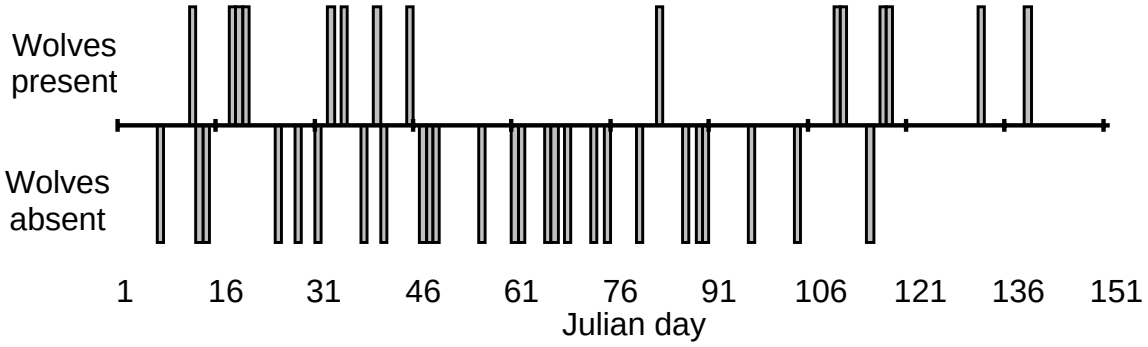


Figure 5.3. A timeline showing periods of wolf presence (upper bars) and absence (lower bars) on one of three sites in the Upper Gallatin in the winter of 2005 as an example of the temporal variation in predation risk experienced by elk in that site.

We collected from 255 bulls and 708 cow/calves, or a ratio of 2.77 cow/calf samples:bull samples (sex could not be determined for 17 samples), similar to the sex ratio of the population from counts (2.68 cow/calf:bull). Annual differences in snow followed regional trends, with snowpack in the mild winter of 2005 (snow index ± SD, 18.5 ± 16.8) only half of that in the normal winters of 2004 and 2006 (Fig. 5.1, 38.3 ± 23.0, $t_{277,450} = 12.41$, $P < 0.001$). Elk diets were strongly affected by snow in several ways. Increasing snow index reduced grazing in both normal and mild winters ($F_{1, 366} = 82.52$, $P < 0.001$ and $F_{1, 223} = 41.19$, $P < 0.001$). Grazing in the mild winter formed a higher proportion of the diet than in normal winters (Table 5.1, $t_{277, 450} = 13.62$, $P < 0.001$) and grazing in spring was higher than grazing in all winters (Table 5.1, $t_{253, 727} = 18.06$, $P < 0.001$). Snow had the strongest effect on the diet in most models (Table 5.2).

Elk Diet Selection in Normal Winters

In normal winters, ANCOVA showed that cow/calf groups decreased grazing in response to wolves (back transformed 95% CI, see Methods: 75.6–83.0%, wolves absent versus 61.6–69.4%, wolves present) while bulls showed no response in grazing (56.3–69.7%, wolves absent versus 60.3–72.1%, wolves present), so the sex × wolf interaction was strong ($F_{1, 366} = 11.67$, $P < 0.001$). Bull elk, however, decreased conifer use by half on days when wolves shared the same site (18.7–30.4%, wolves absent versus 8.4–14.7%, wolves present); in contrast cow/calf groups nearly doubled conifer use (4.4–6.6% wolves absent versus 7.8–11.4% wolves present).

Table 5.2. Standardized coefficients from ANCOVA (winters) and ANOVA (spring) showing snow, wolf presence and elk gender effects on the consumption of four forage types.

Model	Parameter estimates ± SE (P value)			
	Snow	Sex[1]	Wolf[2]	Sex × Wolf
Normal				
Winter				
Grazing	-0.435 ± 0.048 (<0.001)	-0.137 ± 0.047 (0.004)	-0.105 ± -0.046 (0.022)	0.160 ± 0.048 (0.001)
Conifers	0.312 ± 0.048 (<0.001)	0.300 ± 0.046 (<0.001)	-0.050 ± 0.045 (0.271)	-0.261 ± 0.047 (<0.001)
Evergreen shrubs	0.249 ± 0.054 (<0.001)	-0.193 ± 0.052 (<0.001)	0.266 ± 0.051 (<0.001)	-0.045 ± 0.053 (0.394)
Woody stems	0.294 ± 0.056 (<0.001)	-0.020 ± 0.054 (0.715)	0.115 ± 0.053 (0.031)	0.057 ± 0.056 (0.303)
Mild Winter				
Grazing	-0.372 ± 0.058 (<0.001)	-0.546 ± 0.061 (<0.001)	0.310 ± 0.066 (<0.001)	0.149 ± 0.065 (0.023)
Conifers	0.044 ± 0.058 (0.455)	0.604 ± 0.061 (<0.001)	-0.211 ± 0.066 (0.002)	-0.102 ± 0.065 (0.119)
Evergreen shrubs	0.378 ± 0.065 (<0.001)	0.128 ± 0.069 (0.065)	-0.132 ± 0.074 (0.076)	-0.076 ± 0.073 (0.298)
Woody stems	0.322 ± 0.066 (<0.001)	0.159 ± 0.070 (0.023)	-0.141 ± 0.075 (0.062)	-0.015 ± 0.074 (0.840)
Spring				
Grazing		-0.402 ± 0.074 (<0.001)	0.173 ± 0.093 (0.063)	0.114 ± 0.088 (0.198)
Conifers		0.496 ± 0.072 (<0.001)	-0.271 ± 0.089 (0.003)	-0.249 ± 0.085 (0.004)
Evergreen shrubs		0.113 ± 0.078 (0.150)	-0.237 ± 0.097 (0.016)	-0.043 ± 0.092 (0.641)
Woody stems		0.233 ± 0.077 (0.003)	0.034 ± 0.096 (0.726)	0.014 ± 0.091 (0.875)

[1] Coefficient indicates the direction and effect size of male gender on consumption.
[2] Coefficient indicates the direction and effect size of wolf presence on consumption.

Consequently, the main effect of wolves on conifer use in normal winters was weak ($F_{1, 366} = 1.22$, $P = 0.271$) but the sex×wolf interaction was very strong ($F_{1, 366} = 30.34$, $P < 0.001$). These opposing responses would have been missed if the sexes had not been separately examined (Fig. 5.4). The sexes also differed in their consumption of evergreen shrubs in normal winters with cow/calf groups consuming more than bulls (Fig. 5.4, sex effect $F_{1, 366} = 13.76$, P<0.001) but both sexes tended to increase consumption of sagebrush when wolves were present (wolf effect, $F_{1, 366} = 27.13$, $P < 0.001$; cow/calf groups: 3.9–5.4%, wolves absent versus 7.6–10.9%, wolves present; bull groups 2.9–4.2%, wolves absent versus 4.1–6.6%, wolves present). Thus, the sex × wolf interaction did not significantly contribute to evergreen browse consumption ($F_{1, 366} = 0.73$, $P = 0.394$). This wolf effect on evergreen shrub use was stronger than the effect of snow in normal winters (Table 5.2). Wolves also positively (but less strongly than snow) affected consumption of woody stems ($F_{1, 366} = 4.68$, $P = 0.031$)

Elk Diet Selection in a Mild Winter and Spring

Broad diet composition, sex differences, and responses to wolves were very similar between the extremely mild winter of 2005 and the spring of all three years, and diets under both circumstances were quite different from normal winters (Fig. 5.4). Diets were more strongly biased by sex in the mild winter and spring, as cows grazed considerably more than bulls (sex effect, mild winter only, $F_{1,223} = 80.02$, $P < 0.001$; spring, $F_{1,223} = 29.15$, $P < 0.001$). Cows did not decrease grazing in response to wolves in the mild winter, as they had in the normal winters (89.1–92.2%, wolves absent versus 91.7–94.3%, wolves present).

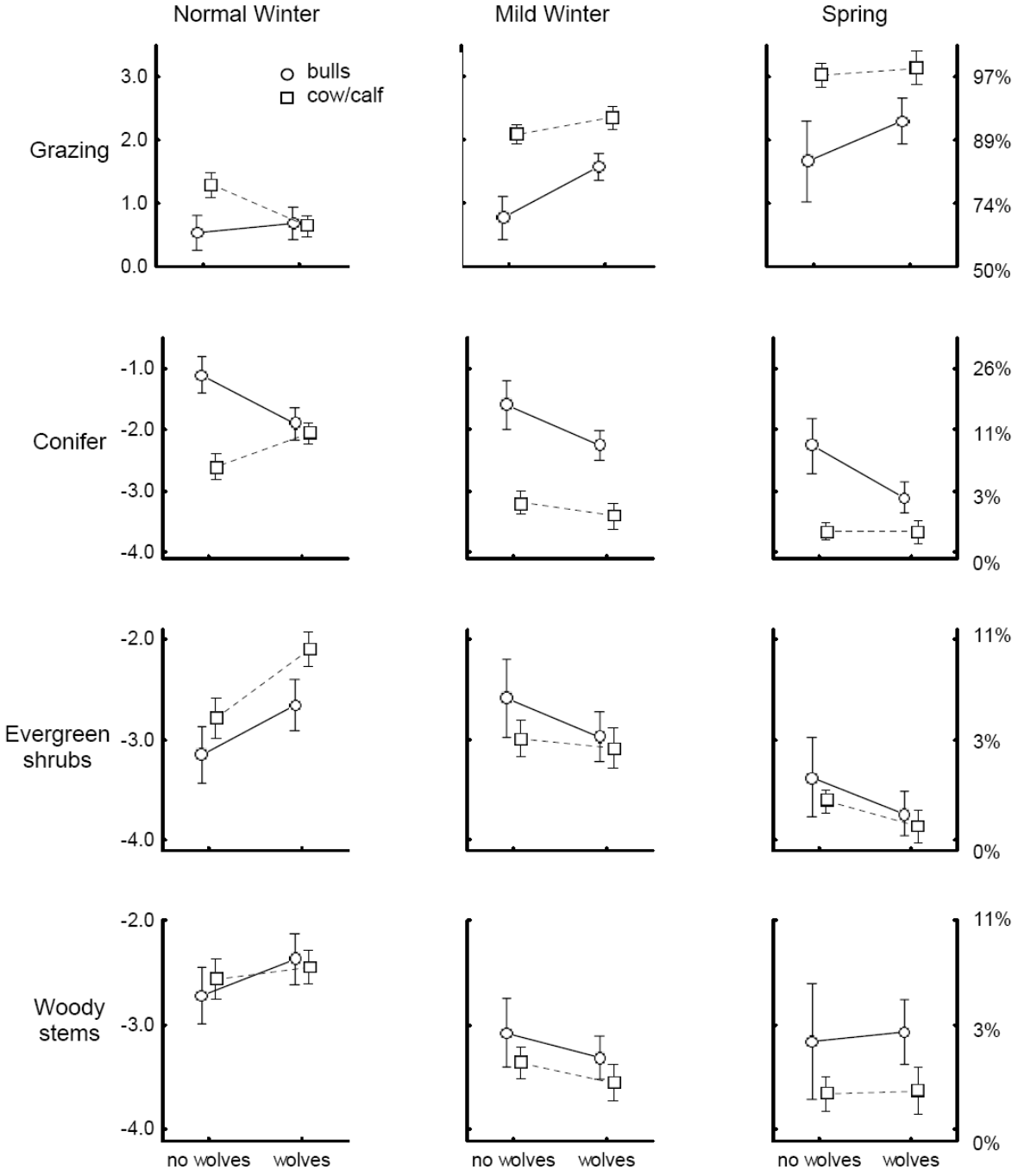


Figure 5.4. Consumption of the proportion of the diet composed of various forage types (mean and 95% CI) in groups of adult male (bull) and adult female (cow/calf) elk in the Upper Gallatin and response to the presence (wolves) or absence (no wolves) of wolves in normal winters, a mild winter, and spring. Left axis shows logit-transformed proportions and the right axis is back-transformed to percentages. Means shown for normal and mild winters are LS means from ANCOVA with snow (the continuous covariate) controlled at its mean

Somewhat surprisingly, bulls increased grazing on days when wolves shared the same site, (60.7–75.7%, wolves absent versus 80.7– 87.3%, wolves present) whereas in normal winters they showed only a weak, nonsignificant response (Fig. 5.4). Consequently, there was no difference in the sign on the sex × wolf coefficient between normal and mild winters (Table 5.2; mild winters, $F_{1,223} = 5.26$ $P < 0.023$), even though the detailed patterns that produced this interaction were quite different (Fig. 5.4). This sex-dependent response in grazing was similar to that observed in spring (Fig. 5.4, cow/calf groups 95.6–96.6%, wolves absent versus 95.8–97.0% wolves present; bulls 74.0–92.7%, wolves absent versus 89.3–95.0% wolves present) though the sex × wolf interaction was weaker in spring ($F_{1,253} = 1.67$, $P = 0.198$). The main effect of wolves on grazing was marginally significant in spring ($F_{1, 253} = 3.49$, $P = 0.063$), and cow/calf groups generally failed to show any responses to wolves in consumption of individual browse components in the mild winter or spring (Fig. 5.4). In response to wolf presence, bulls tended to evenly decrease consumption of conifers and evergreen shrubs in the mild winter of 2005 (conifers: 10.1–22.2% wolves absent versus 5.9–10.0% wolves present and evergreen shrubs: 3.9–8.2% wolves absent versus 3.4–4.8% wolves present) and in spring (conifers: 4.8–12.3% wolves absent versus 3.1–4.3% wolves present and evergreen shrubs: 1.9–3.9% wolves absent versus 0.8–2.7% wolves present).

Discussion

Grazing and browsing are functionally very different foraging strategies, each with unique constraints and nutritional benefits (Clauss *et al.*, 2003; Ngugi *et al.*, 1995).

Empirically derived averages of grass and browse in the diet are often a primary characteristic used in herbivore classification (Hofmann, 1989). Attempts to uncover the significance of herbivores' diets have focused strongly on fixed levels of grazing and browsing, leading to an overabundance of theories (Clauss *et al.*, 2003; Janis *et al.*, 2000; Mysterud *et al.*, 2001; Owen-Smith, 1997; Robbins *et al.*, 1995; Perez-Barberia & Gordon, 2001). Intraspecific variation in the balance of grazing and browsing (and its causes) has received less attention but predator-induced diet shifts on the grazer-browser continuum may have important consequences for nutrition, competitive interactions, trophic cascades, and diversification. Particularly for intermediate feeders, identifying what factors cause individuals to oscillate between these two unique foraging strategies may provide novel insight into herbivore evolution.

Perhaps the simplest generalization of wolf effects on elk diet is that wolf presence led the sexes to consume similar diets. This is important because the sexes were strongly segregated (dietarily) in the absence of wolves and remained spatially segregated in the presence of wolves. Regardless of which sex responded, strongly overlapping diets were the ultimate result of foraging under the risk of predation. Previous research (Winnie *et al.*, 2006) showed that the elk gender and extrinsic factors became weaker predictors of elk habitat selection when wolves were present in the same drainage. Our results show that this behavioral response to risk leads to a more uniform diet across the elk population. This adds further complexity to our current understanding of sexual segregation as we confirmed that factors related to predation risk cannot be disentangled from factors related to forage acquisition (Mcnamara & Houston, 1987) because both were uniquely related in each sex. Perhaps predation risk reinforces spatial segregation

because the presence of wolves would exacerbate asymmetric competition for forage between sexes, a new hypothesis presented by our data. However, because the greatest dietary differences between sexes were seen when constraints on foraging were weakest (Fig, 5.4. no wolves in the mild winter or spring) then physiological or allometric differences may ultimately be driving segregation

Mean diet composition, responses to wolves, and sex differences were strongly mediated by seasonal conditions, most likely through effects of snow on the availability of forage grasses. Our results confirm that snow conditions are extremely important in determining how herbivores use vegetation. Not surprisingly, snow had stronger (negative) effects on grazing in normal winters than in the mild winter, and snow always positively affected the consumption of all types of browse. In the very mild winter all elk showed very high levels of grazing, similar to spring, although spring grazing may also be strongly driven by changes in preference (rather than availability) as nutritious, green grass shoots emerge. The differences in responses to wolves between winters strongly suggest that the effects of climate change on trophic interactions may not be simple linear extensions of trends found in current or historical winters. Decreasing snowpack and warmer temperatures will not only change broad diet patterns in herbivores, but may also alter how herbivores interact with conspecifics and respond to predators.

Finally, willow (*Salix spp.*), aspen (*Populus tremuloides*), and cottonwood (*Populus deltoides*) in the GYE have experienced enhanced growth following wolf reintroduction, and this release has been attributed to a behaviorally-mediated trophic cascade mediated through elk browsing (Morell, 2007; Ripple & Beschta, 2004; Beschta & Ripple, 2007; Ripple & Beschta, 2003). Whether wolves drive this release has been

debated (Kimble, 2007) (Ohman 2008). Direct data on elk consumption of willow (Creel and Christianson in review) suggest that the antipredator behavior of elk actually favors willow suppression, while recent reductions in elk density and snowpack favor willow release. To broaden the focus of this discussion, our results suggest a hypothesis that behaviorally-mediated trophic cascades may be occurring in forage plants such as grasses, conifers, and evergreen shrubs, all of which are far more important to elk than rare food items like willow. For example, if adult females in the Upper Gallatin elk population ($n \sim 720$) reduce grazing from 80% to 66% of the diet on days when wolves are present, each elk consumes 4.5 kg of primary production/day, and wolves are present for 50% of 120 d in winter, then, each winter, approximately 27 fewer metric tons of herbaceous vegetation are being grazed in foraging areas occupied by adult females since wolf reintroduction. Likewise, evergreen shrubs across the winter range are probably experiencing significant increases in winter browsing since the arrival of wolves while conifers are likely experiencing contrasting changes (either more or less browsing) dependent on local patterns of sexual segregation (Fig. 5.4). We currently know little about how these risk effects are shaping important plant communities in areas recolonized by top predators. However, we can conclude that behavioral responses to wolves (Creel *et al.*, 2005; Winnie & Creel, 2007) alter the diets of elk, paralleling changes in reproductive physiology, demography and population dynamics (Creel *et al.*, 2007a). Given the dominant role of elk in this ecosystem, community-wide responses seem likely (Croll *et al.*, 2005; Mclaren & Peterson, 1994). Future research should investigate how these risk effects alter elk nutrition and impact community members.

CHAPTER 6

ELK, SNOW, WOLVES AND WILLOWS: A TEST OF THE BEHAVIORALLY MEDIATED TROPHIC CASCADE HYPOTHESIS

Abstract

Recent increases in the height and growth ring width of willow (*Salix* spp.) in the Greater Yellowstone Ecosystem (GYE) have been attributed to a behaviorally mediated trophic cascade from wolves (*Canis lupus*) to elk (*Cervus elaphus*) to willows. This hypothesis predicts that elk consume less willow in response to the presence of wolves, a prediction that has not been directly tested. We collected 727 fresh fecal samples from elk in the Gallatin Canyon portion of the GYE over three winters, and used microhistological methods to quantify the proportion of willow in each sample. We then tested the effect of wolf presence on willow consumption by elk, controlling for the effects of snow conditions, sex and habitat type (which have been shown to affect the diet of elk). During the period of study, 8-17 wolves hunted within the study area, and wolves were locally present on 49% of the 260 sampling days, stratified at two-week intervals across three drainages. Over the three years combined, willow consumption was significantly related to snow conditions, wolf presence, and a wolf x sex interaction. Willow consumption increased as the snowpack became deeper or less penetrable, and this effect was strong. Willow consumption increased in response to the presence of wolves, contrary to the hypothesis that wolves release willow from elk browsing through behavioral mechanisms. Sex-differences in the consumption of willow were not consistent, and the sexes differed in their response to wolves. To examine a broader

temporal scale, we aggregated the data to estimate winter-long mean willow consumption for each drainage: at this scale, willow consumption also increased as predation risk increased. In summary, willow consumption by elk was more strongly affected by snow conditions than by the presence of wolves. Interactions between elk and willow are affected by wolves, but not in the manner predicted. Recent willow release in the GYE is well explained as a numerically-mediated cascade, reinforced by patterns of snow accumulation.

Introduction

A trophic cascade occurs when the limiting effect of predation spills over from prey to lower trophic levels, for example from carnivores to herbivores to plants. In 1960, Hairston et al. proposed that predators commonly enhance plant productivity or biomass by limiting the density of herbivores (Hairston 1960). The generality of this pattern has been debated (Polis 1999), but subsequent research has shown that cascading effects of predators on plants are common, particularly in aquatic systems and in simple food webs (Shurin et al. 2002). It is debated whether terrestrial predators have effects on plant communities that are as strong as those seen in aquatic systems (Heithaus & Dill 2006), because terrestrial plant communities are typically more complex, and terrestrial plants typically have more elaborate defenses against herbivory (Schmitz et al. 2000, Shurin et al. 2002, Polis 1999). In particular, there are few examples of trophic cascades driven by terrestrial vertebrates, especially mammals (Schmitz et al. 2000), though some clear cases have been described (Croll et al. 2005).

In the Greater Yellowstone Ecosystem (GYE), measurements of plant height, growth ring width and the percentage of stems browsed suggest that willows (*Salix* spp.) and aspen (*Populus* spp.) are being released from herbivory by elk (*Cervus elaphus*) since wolves (*Canis lupus*) were reintroduced in 1995-1996 (Ripple et al 2001, Smith et al. 2003, Ripple & Beschta 2004, Beyer et al. 2007). A similar trophic cascade has been reported in Canada's Banff National Park (Hebblewhite et al. 2005). Currently, there is some consensus that the release of willow in the GYE is due to cascading effects from wolves, and that the cascade is mediated primarily by changes in elk behavior, rather than a reduction in elk numbers (Ripple et al 2001, Smith et al. 2003, Ripple & Beschta 2004, Fortin et al. 2005, Beyer et al. 2007). Beyer et al recently summarized this conclusion as follows: "We found little evidence that reduced elk population size was associated with increased willow growth. Instead, we found better evidence for the presence of wolves on the landscape influencing willow growth, implicating indirect [behavioral] rather than direct [numerical] effects of wolves on elk herbivory of willow". The conclusion that density-mediated indirect effects are relatively unimportant in this system is perhaps surprising because elk numbers have declined by approximately 40% since wolf reintroduction (Creel et al. 2005, 2007, White & Garrott 2005).

The simplest prediction of the behaviorally-mediated trophic cascade hypothesis is that elk should decrease their consumption of willow in the presence of wolves. To date, this prediction has not been tested. Here, we used 727 elk fecal samples from three winters to measure the proportion of the diet comprised of willow when wolves were locally present or absent. We found that elk increased their consumption of willow in response to the presence of wolves, opposing the prediction of the behaviorally-mediated

cascade hypothesis. We found that willow consumption increased as snow accumulation increased, by increasing the difficulty of gaining access to grasses. This effect of snow on grazing/browsing trade-offs has also been observed in many other elk populations (Fig. 6.1, Telfer 1978, Telfer & Kelsall 1984, Jenkins & Wright 1987, Adamczewski et al. 1988, Kay 1997, Zeigenfuss & Singer 2003, Christianson & Creel 2007). By directly testing the effect of wolf presence on willow consumption, and by examining recent changes in elk numbers and snow conditions, we conclude that the recent release of willow in the GYE is well explained as a numerically mediated cascade and as a response to recent patterns of snow accumulation, but not as behaviorally-mediated cascade.

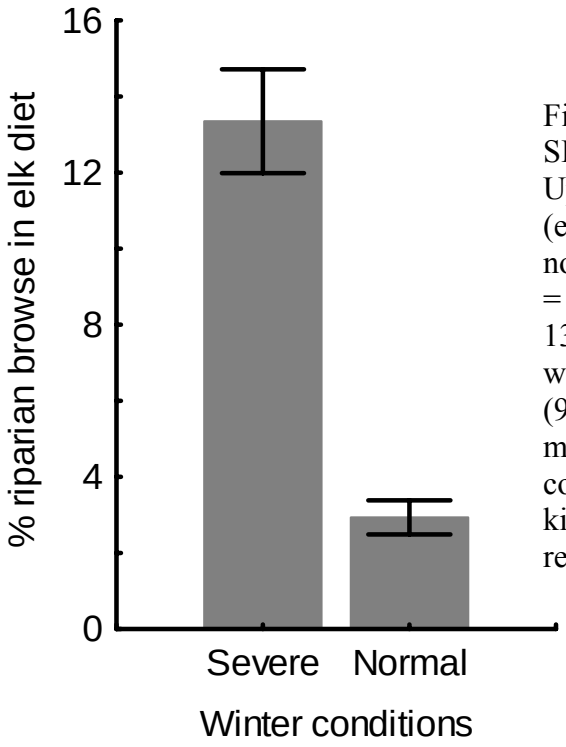


Figure 6.1. Riparian browse consumption (± SE) by elk prior to wolf recolonization the Upper Gallatin during severe winters (extended periods of deep snow with below normal temperatures, leading to starvation, n = 61 diet samples) and normal winters (n = 133). Riparian browse includes several woody plant species but is primarily willow (92%, by volume). Data come from macroscopic analysis of stomach contents collected from winter-killed and hunter-killed animals (data from unpublished reports, Montana Fish Wildlife and Parks).

Study Area and Methods

Data Collection

The Upper Gallatin elk herd (1100 ± 260 animals, mean ± SD, from aerial counts in 2003-2005 by Montana Fish Wildlife and Parks) occupies several wilderness areas in southwestern Montana and Yellowstone National Park, Wyoming, USA. Around November, these elk migrate annually from mountainous, alpine summer ranges (>2400 m ASL) to lower elevation winter ranges (1975-2200 m ASL) along the upper Gallatin river. Although willow covers less than 2% of the regional landscape (Houston 1982), habitats along the river, its tributaries, and other moist sites in the study area are often dominated by willow (Patten 1963, Patten 1968, Shoutis 2007). Recent and historical data confirm that the elk population consistently distributes itself in winter across three primary winter ranges (Fig. 6.2), consisting of 4 drainages of the Upper Gallatin River (Creel et al. 2005). To representatively sample the Gallatin elk population, we stratified our data collection across these three sites. We counted and classified (by age and sex) elk in each drainage by walking a fixed transect in each drainage approximately every 14 days from December – March 2004 – 2005 and December – April 2006. We recorded the composition and location of every elk group seen, and the habitat type at their location (conifer forest, sagebrush-steppe, grassland, or riparian habitat). We also recorded all elk seen opportunistically while collecting other data on elk foraging behavior, wolf predation, and environmental conditions throughout the winter (Creel & Winnie 2005, Winnie & Creel 2007 – see also for further description of the study area and general methods).

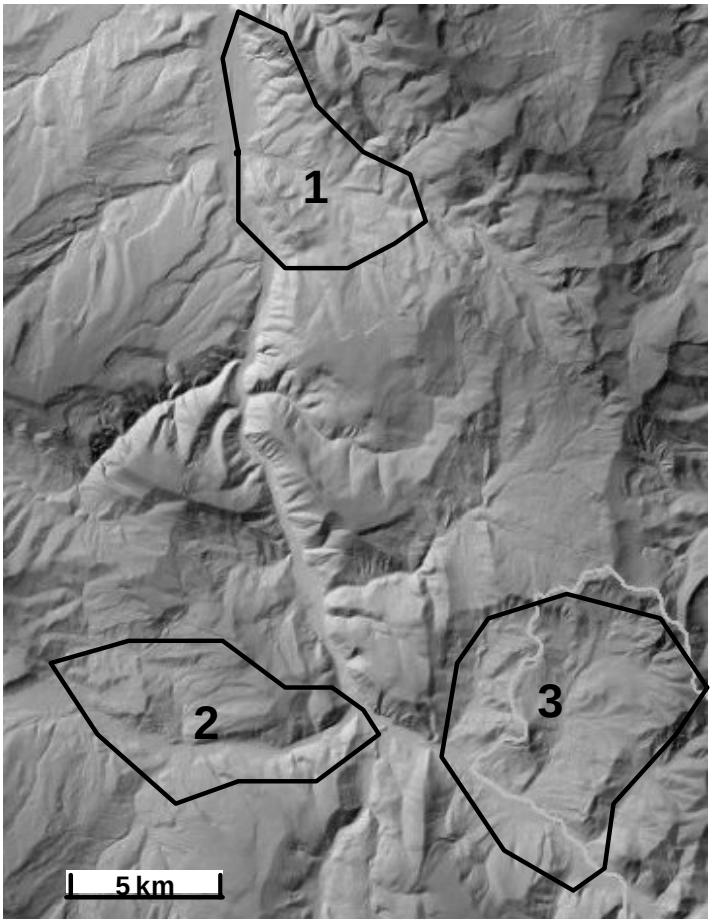


Figure 6.2. Study area in the Upper Gallatin in southwestern Montana, USA. Boundaries of the primary winter study areas are outlined on a base map showing the local topography. The Gallatin River runs south to north through the middle of the figure and the northwest corner of Yellowstone National Park (light gray line) projects into site 3.

Riparian Browse Consumption: At two-week intervals, we collected 3–10 fresh (<24 hrs) fecal samples (~ 30 ml) from each of the three sites. We always sampled from a single group of elk ($\bar{X}$ = 8.8 individuals sampled per collection), and each sample camfrom a single individual (assessed by direct observation or by snow tracking). We also collected rumen and fecal samples opportunistically from elk killed by wolves and from other elk carcasses found on the study site (primarily mortalities from vehicle

collisions). Beginning in 2005, we noted the habitat type at all fecal collection points as conifer forest, sagebrush-steppe, grassland, or riparian. We used microhistology to determine the botanical composition of each sample (Sparks & Malechek 1968) by preparing microscope slides of epidermal plant tissue from feces or stomach samples that had been washed, bleached, and stained. We identified fragments by comparison to reference slides, identifying a minimum of 50 fragments per sample for each of 727 samples, after confirming that 30 fragments yielded good estimates of diet composition. We categorized fragments according to broad vegetative groups, including graminoids, forbs, conifers, sagebrush (*Artemeisa* spp.), woody stems, and a willow/aspen category. Because the epidermal features of willow and aspen are similar, the microhistological technique itself did not distinguish between the two species, but willow is more common than aspen by 2-3 orders of magnitude on our study site (Shoutis 2007, Kimble 2007 Patten 1963, 1968), and previous research has shown willow to be 40 to 90 times more abundant than either aspen or other riparian browse plants in the diets of Gallatin elk (willow mean ± SD, 2.8 ± 5.2% of diet, $n = 133$, unpublished data, Montana Fish Wildlife and Parks, Bozeman, Montana, USA). Finally, aspen has been suggested to respond to wolf reintroduction through a trophic cascade in the same manner as willow (Ripple & Beschta 2007). For these reasons, we refer to this category simply as riparian browse or willow.

Variation in Predation Risk: Wolves recolonized the study area in 1996 following reintroduction in Yellowstone National Park (Fritts et al. 1997). During this study, 8-17 wolves in 2 - 3 packs (with 0-4 radiocollars) moved in and out of each drainage, creating

temporal variation in local predation risk for elk. While walking fixed transect routes, and during daily visits to drainages to collect other types of data, we continuously checked for signs that wolves were present within that drainage on that day. We considered wolves present within a drainage if we located them via VHF radiotelemetry, found a fresh kill, fresh scat, or fresh tracks in snow, mud or loose soil. This description of fine-scale temporal and spatial variation in the risk of predation by wolves has shown power to detect effects of risk on elk grouping patterns, habitat selection, vigilance and foraging behavior and we predicted that it would also detect differences in willow consumption due to predation risk, if they exist (see Results).

The hypothesis of a behaviorally-mediated trophic cascade predicts that willow consumption should decline on days that wolves were present. Our primary method for testing this prediction is based on fresh fecal samples, which represent the foraging decisions made 20-60 hrs in the past (Jiang & Hudson 1996). Because of this long gut-passage time, it is possible that the presence or absence of wolves on the day of fecal collection might not describe predation risk at the time that the elk harvested the plants within the feces. To address this issue, we considered a diet sample to represent foraging decisions with wolves present if wolves were detected within that site on the day of, the day preceding, or two days preceding fecal collection. We considered a diet sample to represent foraging with wolves absent if wolves were not detected within that site on any of those three days.

Variation in Local Snow Conditions: Snow conditions are the best-documented factor affecting the consumption of willows and other woody plants by elk (Telfer 1978,

Telfer & Kelsall 1984, Jenkins & Wright 1987, Adamczewski et al. 1988, Kay 1997, Zeigenfuss & Singer 2003, Christianson & Creel 2007). Elk diets generally show a strong bias toward grazing, but browsing increases when snow conditions restrict their access to grass. The depth of snow alone does not fully describe constraints on grazing. Crusted, wind-packed, or trampled snow interacts with depth to limit access to grass (Skogland 1978, Telfer & Kelsall 1984, Sweeney & Sweeney 1984). We recorded snow depth and compaction during biweekly elk transects at 3 fixed locations in each drainage in open areas used by elk for foraging (Winnie et al. 2006). To measure snow compaction, we dropped a 3 kg spherical steel ball from a height of 30 cm and we recorded its penetration, along with total snow depth to the nearest 1 cm. We converted this to a proportion, as follows: *compaction* = 1 – (*cm of penetration*/*cm of total snow depth*). This proportion scaled the range from loose powder snow (0) to solid crust (1). Preliminary research established that a drop height of 30 cm matched the depth of penetration for fresh elk tracks at the same location, and revealed this to be a sensitive method to quantify the manner in which snow conditions affect the behavior of elk (Winnie et al. 2006, Liley & Creel 2007).

To reduce parameters (see Data Analysis) we combined snow depth and compaction into a single index to describe the constraint on elk grazing (and thus, the incentive for elk to browse). For each 14-d period, we combined the minimum snow depth with the maximum compaction in each drainage using the formula, *snow index*= $snow\ depth_{min} + snow\ depth_{min} * compaction_{max}$. We used the minimum snow depth because elk preferentially select areas of little snow (Houston, 1982, Winnie et al. 2006). In mixed landscapes of conifer and open meadows (as on our study area), snow remains

deep in some areas while open ridges are blown clear and south-facing slopes melt out, both becoming preferred elk foraging areas (Creel et al. 2005).

Sex: Male and female elk differ in social grouping, habitat selection, activity budgets, movements, life history strategies, sensitivity to environmental conditions, and, importantly, foraging strategy (Clutton-Brock et al. 1982, Winnie et al. 2007, Leege & Hickey 1977, McCorquodale 2003). Elk have always been strongly sexually segregated within the study area (Peek & Lovaas 1968) with males being most common in the south-eastern portion (site 3: 71% of 4035 elk in 662 groups were adult males) and adult females with calves distributed across the remainder of the winter range (sites 1 & 2: 3% of 9069 elk in 476 groups were adult males) during the study period. Within single drainages, elk groups were also strongly segregated, and 92% of all elk were in groups with ≥90% or ≤10% males (Fig. 6.3).

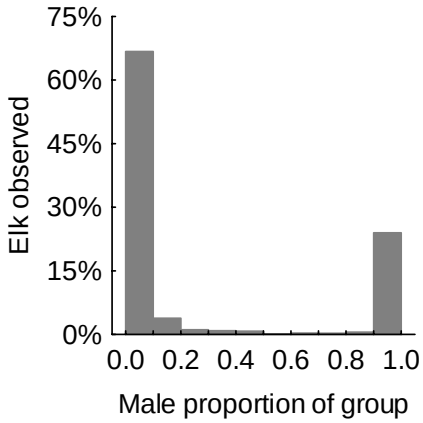


Figure 6.3. Sex bias of elk groups in the Upper Gallatin in winter. Histogram shows the frequency at which elk are found in groups with the given proportions of adult males.

We used a combination of the following information to classify fecal samples according to sex, 1) the sex bias of the observed group from which feces was collected, 2) the sex bias in the drainage at the time of each fecal collection, and 3) fecal pellet size (Maccracken & Van Ballenberghe 1987, Khan & Goyal 1993). If the group was directly

observed before fecal collection and the proportion of adult males was >85% or <15%, we classified samples as 'bull' or 'cow/calf", respectively, for those samples (n = 195 or 26.82%). If groups were not directly seen before fecal collection, but the 3 most recent drainage composition counts revealed a male bias of >85% or <15% than we classified sex as 'bull' or 'cow/calf' respectively (n = 445 or 61.21%). If the group or drainage composition was of mixed sex (85% ≥ % male ≥ 15%) we used pellet size to distinguish between the sex of individuals (n = 78 or 10.73%). Sex could not be determined using any of these methods for 9 samples (1.2%). Sex-specific pellet size was determined from probability density functions for pellet size developed for calves, adult females, and adult males using fecal pellets from individuals of known age/sex (n = 83, Fig. 5.2).

Year: Despite efforts to quantify the relevant independent variables, inter-annual variation in unmeasured factors can significantly alter elk behavioral responses to predation (Gude et al. 2006). To address this issue, we examined riparian browse consumption separately for each year, and in combination (2004, 2005, 2006 or all) A restricted-entry hunting season occurred on the study area in sites 1 and 2 during the period of data collection in 2004 and human hunting has been shown to influence willow consumption in elk (Morgantini & Hudson 1985). To consider whether this might have affected inferences, we excluded samples collected from sites 1 and 2 in 2004 from some analyses, identified in the Results.

Statistical and Analytical Methods

Prior to analysis, we standardized all variables to allow direct comparison of effect sizes. Because the dependent variable (proportion of the diet comprised of willow)

was biased toward values of zero, we logit-transformed it prior to analysis (Fox 1997). We could not fit the model for a full factorial design including all independent variables and their interactions, because some effects could not be estimated. Consequently, we used analysis of covariance (ANCOVA 1) within each year to test the effects on willow consumption of wolf presence, elk sex and the snow index. We also used analysis of covariance (ANCOVA 2) with data from 2005 and 2006 to test for interactions between of wolf presence and habitat type on riparian browse consumption, while controlling for snow conditions (recall that the local habitat from which fecal samples were collected was not recorded in 2004).

To test for effects of predation risk on willow consumption at a longer time scale, we collapsed the data on wolf presence to give a winter-long measure of the frequency of wolf presence (i.e. proportion of days in each winter-site combination site with wolves present). We used this measure of predation risk as a categorical variable in ANCOVA 3, to examine the effect of the site-year (each differing in the background level of risk) on riparian browse consumption while controlling for the snow index. We also regressed riparian browse consumption onto the winter-long frequency of wolf presence within each site-year (now treated as a continuous variable rather than categorical as in ANCOVA 3) and snow conditions to provide a final estimate of the effect of wolves.

We conducted this overlapping but complementary set of tests to be cautious about the possibility of idiosyncratic results. Consistency of effects across complementary analyses enhances the strength of the inferences.

Results

Wolf Presence and Use of Riparian Habitat by Elk

Wolves were detected on 49% of 260 sampling days, when averaged across sites and years (Table 6.1). On these days, we observed 11826 elk, with 50.8% counted on days that wolves were present (Table 6.1). In general, we observed few elk in riparian habitats (7.3% of the total), and detected no difference in the number of elk in riparian areas between days when wolves were present or absent ($\chi^2 = 0.16$, $P = 0.685$). There was a weak tendency for elk to be detected in riparian areas more frequently on days when wolves were present ($\chi^2 = 2.27$, $P = 0.132$, Table 6.2), but in smaller groups (mean ± SD, 10.6 ± 15.1 versus 16.6 ± 27.9, respectively, $t = -1.09$, df = 60 $P = 0.278$).

Table 6.1. Number of days with wolves present or absent at each site. The number of elk observed is given in parentheses.

	Site			
Wolves	1	2	3	Total
Absent	45 (2478)	44 (2057)	45 (1281)	134 (5816)
Present	41 (3112)	19 (547)	66 (2351)	126 (6010)

Table 6.2. Number of days with elk detected in riparian habitat and in other habitat types with wolves present or absent. The number of elk observed is given in parentheses.

Wolves	Riparian	Other Habitats	Elk not seen
Absent	17 (382)	173 (5305)	6
Present	29 (415)	182 (5594)	8

Snow, Wolves and Willow Consumption by Elk

We determined willow consumption for 727 elk fecal samples. Most samples (66.9%) contained no willow ($n = 486$), and average (± SE) willow consumption was low

(2.47% ± 0.21%) but varied considerably (range 0 – 60%). This low mean and high variance in willow consumption is typical of elk populations under most environmental conditions (Christianson & Creel 2006).

ANCOVA 1: In 2004 and 2005, willow consumption was not detectably affected by wolf presence (see Table 6.3 for effect size & statistics, this and subsequent tests). In 2006 (when predation risk was greatest) and for all years combined, elk consumed significantly more willow when wolves were present. These results are contrary to the behaviorally mediated trophic cascade hypothesis, which posits that elk avoid willow in response to wolf presence (Table 6.3). Deep, compact snow increased willow consumption in 2004 and 2005 but did not detectably affect willow consumption in 2006. Predation risk was highest in 2006, and predation risk has been shown to constrain the responses of elk to variation in snow conditions (Winnie et al. 2006). Sex had no effect on willow consumption in 2005 and had effects in opposing directions in 2004 and 2006 (Table 6.3). Males consumed more willow than females when snow had a strong effect on the diet (2004, see Fig. 5.1), and less willow than females when wolves had a strong effect on the diet (2006).

A parallel analysis that excluded samples collected in areas with human hunting (sites 1 and 2 in 2004) confirmed positive effects of snow depth/compaction and wolf presence on riparian browse consumption, with an interaction between elk sex and wolf presence. Bull groups showed a stronger increase in riparian browse consumption in response to wolves than cow/calf groups did (Table 6.3, analysis with years combined).

Table 6.3. Results from ANCOVA of willow consumption by elk as affected by snow conditions, wolf presence and elk sex, for three winters and in combination. Variables were standardized so effect sizes can be compared directly.

Year	Parameter Estimates ± SE (P value)[1]				*n*	*F* (*P*)
	Snow	Sex[2]	Wolf[3]	Wolf*Sex		
2004	**0.254 ± 0.088 (0.004)**	**-0.241 ± 0.093 (0.011)**	-0.099 ± 0.081 (0.223)	0.094 ± 0.092 (0.311)	192	4.60 (0.001)
2005	**0.467 ± 0.062 (<0.001)**	0.058 ± 0.066 (0.378)	-0.049 ± 0.071 (0.492)	0.004 ± 0.070 (0.957)	227	15.18 (<0.001)
2006	0.055 ± 0.080 (0.495)	0.211 ± 0.117 (0.072)	**0.191 ± 0.077 (0.014)**	-0.030 ± 0.115 (0.796)	177	3.55 (<0.001)
All[4]	**0.307 ± 0.045 (<0.001)**	-0.019 ± 0.046 (0.675)	**0.152 ± 0.044 (0.001)**	**0.109 ± 0.046 (0.019)**	491	15.20 (<0.001)

ANCOVA 2: In 2005 and 2006, we recorded the habitat type (riparian, other) at the site of each fecal collection for 405 samples (Fig. 6.4). In general, ANCOVA models fit considerably better when habitat type was included in the model and elk sex was omitted ($r^r_a = 0.230$, $F_{4,401} = 31.17$, $P < 0.001$ versus $r^r_a = 0.070$, 0.199, 0.054, and 0.104 for 2004, 2005, 2006 and all years combined, respectively, Table 6.3). As before, deeper/more compact snow increased willow consumption ($b \pm SE = 0.276 \pm 0.048$, $F_{1, 401} = 33.48$, $P < 0.001$), as did wolf presence ($b \pm SE = 0.321 \pm 0.079$, $F_{1, 401} = 16.52$, $P < 0.001$). As expected, riparian browse comprised a larger proportion of fecal samples collected in riparian habitats ($b \pm SE = 0.191 \pm 0.052$, $F_{1, 401} = 13.40$, $P < 0.001$). There was a positive interaction between the effects of wolf presence and riparian habitat ($b \pm SE = 0.297 \pm 0.082$, $F_{1, 401} = 13.24$, $P < 0.001$): elk fecal samples collected in riparian areas contained 5.9 times more willow when wolves were present, when compared to samples from riparian areas with wolves absent (Fig. 6.4: ANCOVA restricted to riparian habitats, $F_{1, 37} = 5.846$ $P = 0.021$).

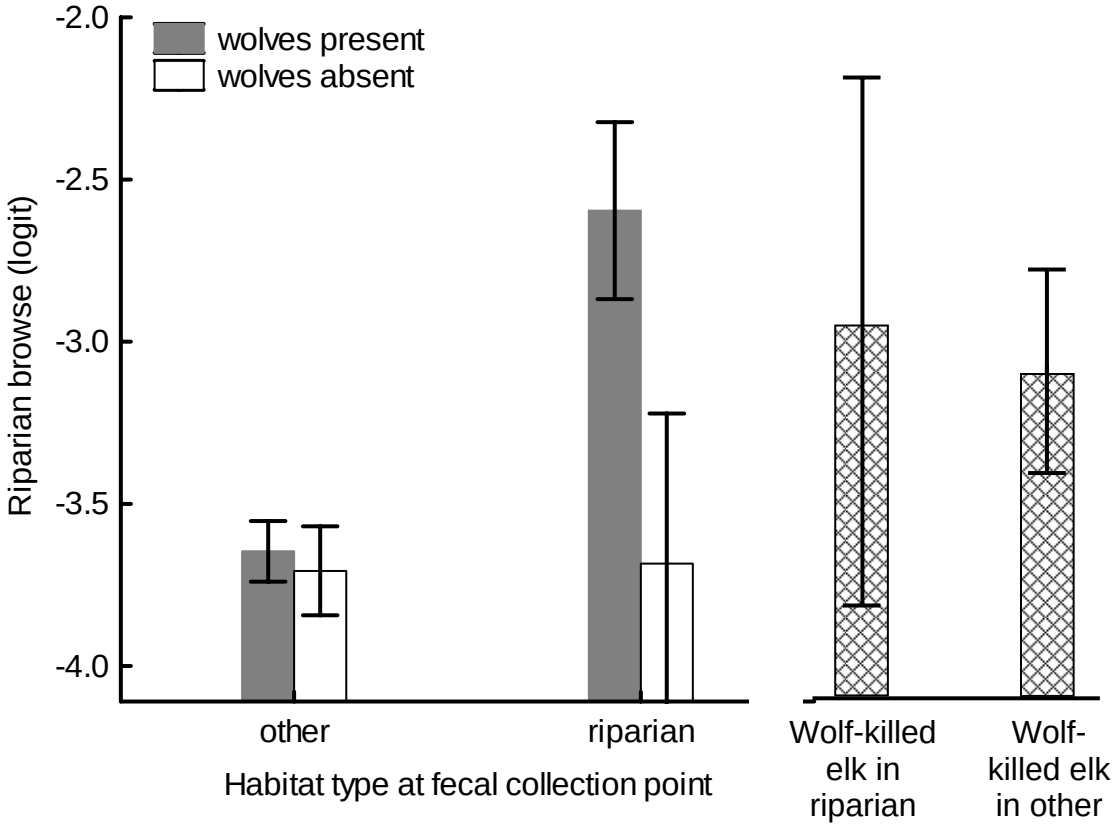


Figure 6.4. Least squares means (± 95% CI) of riparian browse consumption from an ANCOVA considering the effect of snow conditions (here fixed at its mean), wolf presence, and the habitat at the site of fecal collection for 2005-2006. Mean (± 95% CI) riparian browse concentration from stomachs of wolf-killed elk found in riparian and other habitats shows do not differ detectably.

The limited number of samples from killed elk limits the following inferences, but diet samples from wolf-killed elk in found in riparian areas (n = 13) did not differ in willow content from stomachs of wolf-killed elk found in other habitats (n = 43, t_{54} = 0.31, P = 0.762, Fig. 6.4). Elk fecal samples collected in riparian areas when wolves were present did not differ in riparian browse concentration from stomach samples of wolf-killed elk found in riparian areas. (t_{41} = 1.452, P = 0.154). Finally , stomach samples collected from wolf-killed elk found in all other habitat types showed slightly

higher concentrations of riparian browse than fecal samples collected from other habitats when wolves were present ($t_{284} = 3.23$, $P = 0.001$).

ANCOVA 3 & Regression: As a final test, ANCOVA to test for variation among site-years while controlling for snow conditions again confirmed a positive effect of snow depth/compaction on willow consumption (0.248 ± 0.053, $F_{1, 470} = 21.93$, $P < 0.001$), and detected variation among site-years ($F_{6, 470} = 6.22$, $P < 0.001$) that is clearly related to variation in wolf presence. Controlling for the effects of snow, willow consumption showed a consistent pattern of increase as site specific wolf frequency increased across site-years (Fig. 6.5, $r^2_a = 0.124$, $F_{7, 407} = 10.65$, $P < 0.001$). When we regressed willow consumption onto the proportion of days that wolves were present and snow conditions, we detected comparable effects of both snow (0.225 ± 0.40, $F_{1, 576} = 32.01$, $P < 0.001$) nd wolves (0.227 ± 0.40, $F_{1, 576} = 32.59$, $P < 0.001$, $r^2_a = 0.117$, $F_{2, 576} = 39.31$, $P < 0.001$).

Discussion

Our data do not support the primary prediction of the behaviorally-mediated cascade hypothesis. Wolf presence was associated with changes in the consumption of willow by individual elk, but the foraging responses of elk favored willow suppression rather than release (Fig. 6.6). The observed increase in willow consumption when wolves were present is consistent with the observation that elk graze less and browse more in response to human hunting (Morgantini & Hudson 1985). Gallatin elk shifted out of meadows into coniferous forest when wolves were present, possibly seeking refuge as predation risk was higher in areas without cover (Creel et al. 2005. Kauffman et al 2005).

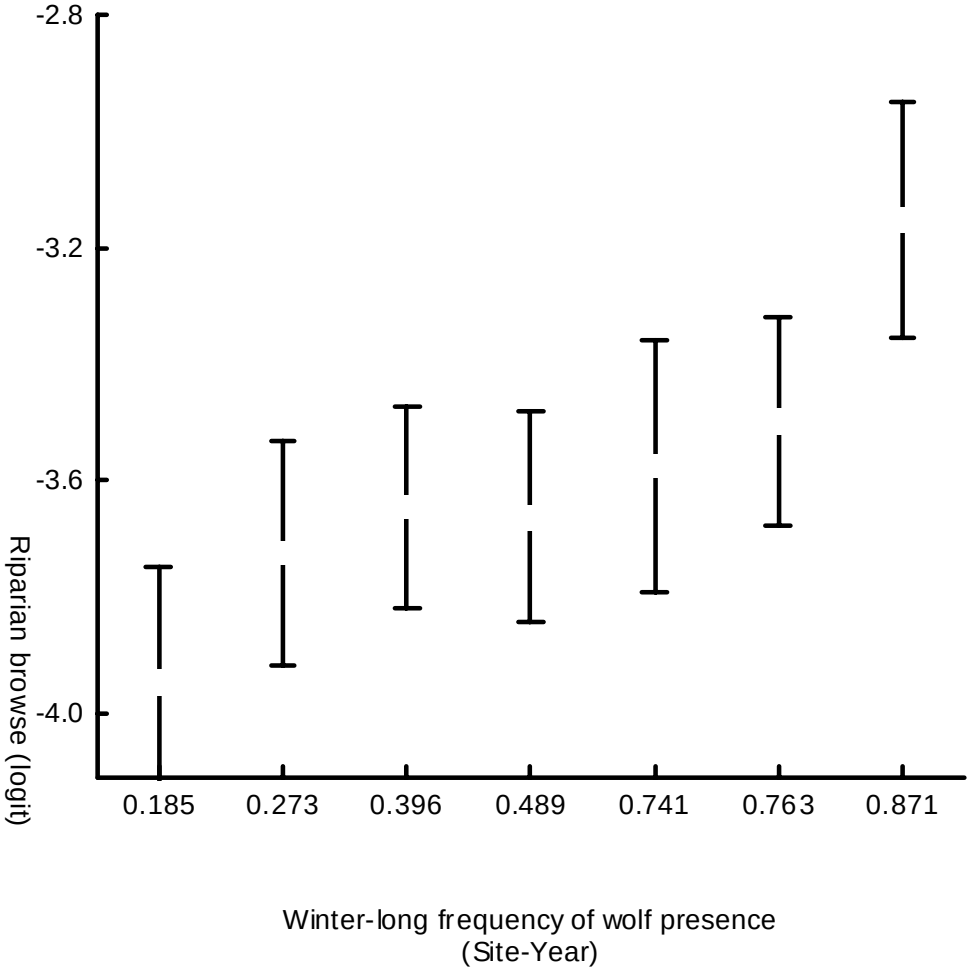


Figure 6.5. Least squares means (± 95% CI) of riparian browse consumption from an analysis of covariance considering the effect of snow conditions (here fixed at its mean) and winter-long frequency of wolf presence at three sites for three winters. Site-year combinations are given in parentheses. Sites 1 and 2 from 2004 are not included because they included human hunting of elk, which is known to affect browsing levels. The ordinate is logit scaled, with the minimum representing ~0% riparian browse.

This analysis of habitat selection in response to risk did not directly test whether elk shifted into willow thickets (because willow constitutes less than 2% of the study area), but dense willow stands may offer protective cover in a manner similar to coniferous forest. It is also possible that elk simply move to the nearest trees or shrubs when they detect wolves, even if the cover is less than ideal, if this response makes them less conspicuous than would be in open meadows. Given that the behavioral responses of elk to wolves increase the consumption of willow, why have willow in the GYE shown increased growth since wolf reintroduction (Beyer et al. 2007)? We suggest that willow release is well-explained by recent patterns of snow accumulation and changes in elk numbers. In many ecosystems (including the GYE), elk show a strong preference for grazing if access to grass is not constrained by snow conditions (Telfer 1978, Telfer & Kelsall 1984, Jenkins & Wright 1987, Adamczewski et al. 1988, Kay 1997, Zeigenfuss & Singer 2003, Christianson & Creel 2007). Willow constitutes a small proportion of the diets of most elk populations (including the Gallatin population), and willow is a large part of the diet only when snow accumulation increases the difficulty of making craters in the snow to gain access to grazing sites (Fig. 6.1). Consistent with this well documented pattern, our data show that willow consumption by Gallatin elk decreased as the depth or compaction of snow decreased.

This result leads to the general prediction that prolonged periods of low snow accumulation should promote the release of willow from elk browsing. Figure 6.6 shows snow accumulation for the two portions of the GYE where willow release has been reported, in the Gallatin Canyon (Ripple & Beschta 2004) and the Northern Range (Smith et al. 2003, Beyer et al. 2007).

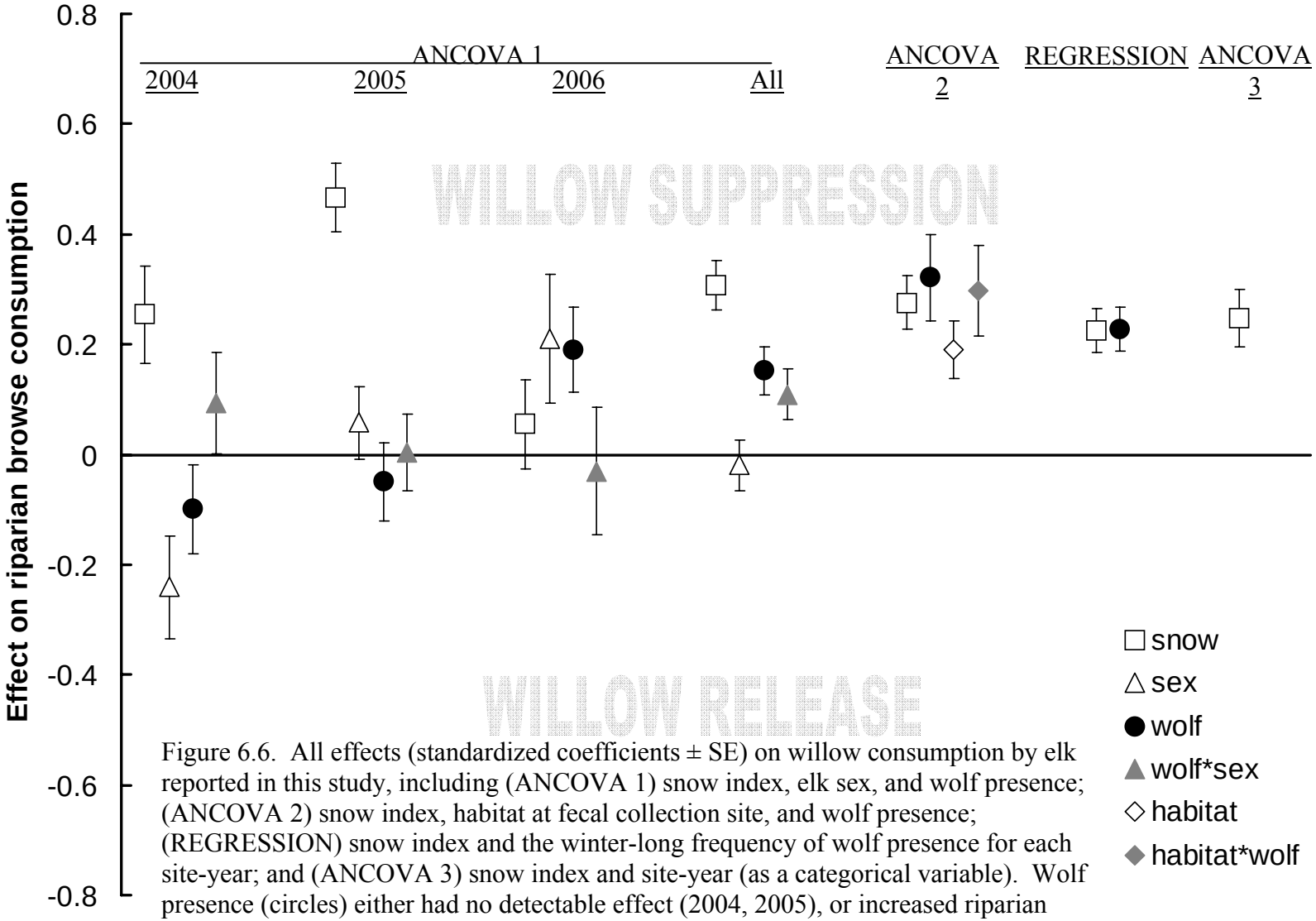


Figure 6.6. All effects (standardized coefficients ± SE) on willow consumption by elk reported in this study, including (ANCOVA 1) snow index, elk sex, and wolf presence; (ANCOVA 2) snow index, habitat at fecal collection site, and wolf presence; (REGRESSION) snow index and the winter-long frequency of wolf presence for each site-year; and (ANCOVA 3) snow index and site-year (as a categorical variable). Wolf presence (circles) either had no detectable effect (2004, 2005), or increased riparian browsing, contrary to the hypothesis of a behaviorally-mediated cascade.

On both of these sites, there were three winters of unusually heavy snow accumulation at the time of wolf recolonization, followed by eight years of relatively light snow accumulation (Fig. 6.7). In the Gallatin Canyon, snow water accumulation for the winters of 2000 – 2007 was 59.9% of accumulation in 1995 – 1997. In the Northern Range, 2000 – 2007 snow water accumulation was 72.4% of accumulation in 1995 -1997. For both sites, the three winters of 1995 – 1997 had the heaviest accumulation of snow in a span of at least 28 years. Combined with our results, this sequence of snow accumulation suggests that willow should have been heavily browsed by 1997, followed by a period of decreased browsing.

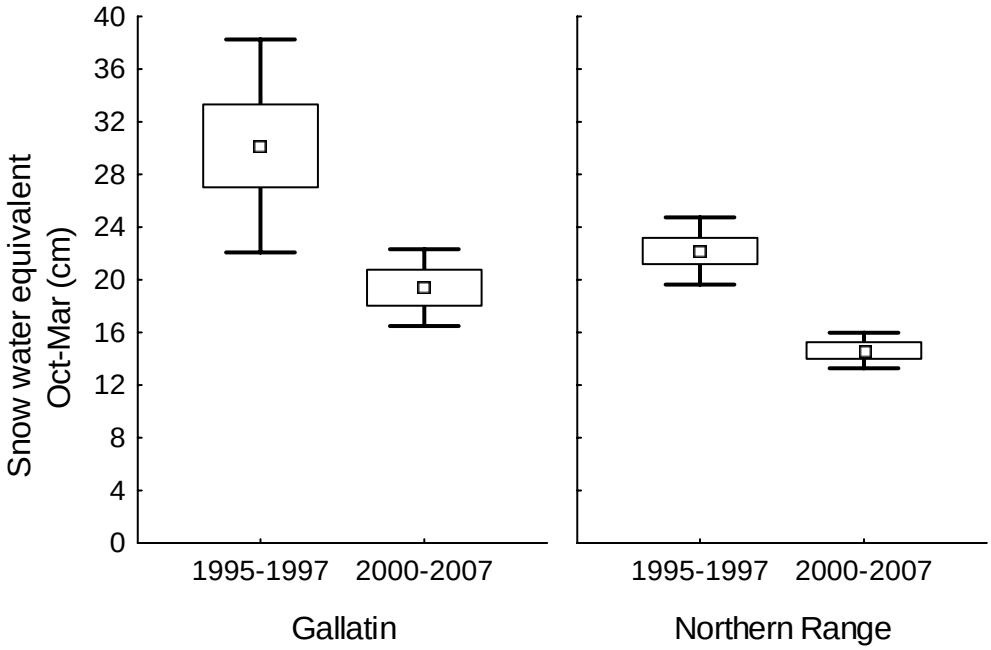


Figure 6.7. Snow water equivalent (mean, ± SE, ± 95% CI), October – March for three winters at the time of wolf reintroduction (1995-1997) and recent winters (2000-2007) for which a cascading release of willow has been reported for the Northern Range of Yellowstone National Park, Wyoming and the Upper Gallatin of southwestern Montana, USA.

In both the Gallatin Canyon and the Northern Range, elk numbers have declined significantly since wolf recolonization (White & Garrott 2005, Creel et al. 2005, 2007). On our study area, 8 aerial counts since wolf reintroduction have averaged 65% of 42 aerial counts from 1929 to 1995. The simultaneous decrease in elk numbers on the Northern Range has been comparably large (White & Garrott 2005), and these declines occurred during a period of mild winters that allowed sustained growth in most of Montana's elk populations (Hamlin 2004). Declines in GYE elk numbers support the hypothesis of a numerically-mediated effect of wolves on willow. Hebblewhite *et al*. (2005) provided evidence for such a numerically-mediated cascade in Banff National Park, though the area of low wolf predation in that study was centered on a town, making it difficult to disentangle the effects of wolves and people as top predators. In constrast, recent studies in the GYE have concluded that the evidence for a numerically-mediated cascade is weak, relative to the evidence for a behaviorally-mediated cascade.

Beyer et al. (2007) used Akaike's information criterion (AIC_c) to compare a set of general linear models of willow growth on the Northern Range of the GYE, using a set of independent variables that included elk counts and a dichotomous variable that coded years as 'wolves absent' (prior to 1996) or 'wolves present' (after 1996). Because models including this wolf variable had better (lower) AIC_c scores than models that included elk numbers, they concluded that there is "little evidence that reduced elk population size was associated with increased willow growth. Instead, we found better evidence for the presence of wolves on the landscape influencing willow growth, implicating indirect [behavioral] rather than direct [numerical] effects of wolves on elk herbivory of willow". This inference does not acknowledge the strong collinearity

between elk numbers and wolf presence in the Northern Range. As their Figure 6.2 shows, the dichotomy "years with wolves absent or wolves present" is virtually identical to the dichotomy "years with more than 15,000 elk or less than 15,000 elk" (only one point changes classification). Given that the dichotomous variable nominally considered 'wolf presence' could equally well have been named 'small elk population', these data can not truly differentiate between numerical and behavioral cascades. We avoided the problem of collinearity in annual changes in the independent variables by examining the response of elk diets to variation in predation risk and snow conditions on a daily time scale. Because wolves come and go frequently on this time scale, predation risk and snow conditions varied independently. Moreover, this approach tests the dietary responses of elk to risk on the time scale at which behavioral decisions are made.

Ripple & Bechsta (2004) also concluded that the effects of wolves on willow arise mainly through a behaviorally mediated cascade. They used a non-statistical, qualitative examination of historical records and photos of willow in the Gallatin Canyon to conclude that "the lower elk population alone probably did not cause the willow release since elk numbers in the Gallatin have been declining for decades yet there was no evidence for willow release until wolves came back into the system". Contrary to these arguments, data from Montana Fish Wildlife and Parks (included in Ripple & Bechsta 2004 as Fig. 6.2b) show that elk counts were increasing, not decreasing, in the period prior wolf recovery (for 1970 – 1995, $b = 50.7 \pm 16.6$ SE, Wald statistic = 9.29, $P = 0.002$; for 1950 – 1995, $b = 8.20 \pm 6.50$ SE, Wald statistic = 1.57, $P = 0.21$). Moreover, the sequence of four willow photographs that Ripple & Beschta used to describe trends in the distribution and height of willow contains a gap of 40 years from 1963 to 2003, with

no data to directly support the conclusion that willow release did not begin until after wolf recolonization in 1996. Pittman Robertson reports by Montana Fish Wildlife and Parks state that willow recovery in several parts of the Gallatin Canyon began much earlier. For example, the 1967 report states: "Willow bottoms in the Taylor Fork and Yellowstone Park areas were beginning to show readily visible recovery over that observed in previous years." The 1964 report states: "Largely due to snow conditions, willow in the Taylor Fork, Snowflake Springs and Monument Creek area was not excessively used." In several Pittman Robertson reports from 1964 onward, parallel statements ad data suggest that willow began recovering prior to wolf reintroduction, in a manner related to snow accumulation.

In summary, Gallatin elk consume more willow when the immediate risk of wolf predation is high. This increase in willow consumption parallels antipredator responses by elk in their habitat selection, behavior and group size (Fortin et al. 2005, Childress & Lung 2003, Creel et al. 2005, Creel & Winnie 2005, Winnie & Creel 2007, Liley & Creel 2007). These responses are also associated with changes in reproductive physiology, demography and population dynamics (White & Garrott 2005, Creel et al. 2007). Because the presence of wolves is associated with an increase in willow consumption, our data do not support the hypothesis of willow release through a behaviorally mediated cascade. In contrast, our data and other recent studies in the GYE support the hypothesis of a numerically mediated cascade mediated by patterns of snow accumulation.

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APPENDICES

APPENDIX A

SUMMARY OF ELK WINTER DIET STUDIES INCLUDED IN OUR ANALYSES

Publication	Location	Range type	Winter	Hunted	Method[1]	Sympatric species[2]
Anderson et al. 1956	Teton area, WY	open	normal	no	R	
Baty 1995	Blackfoot Clearwater WMA[3], MT	open	normal	no	F	WTD, MD
Bergstrom 1964	Yellowstone National Park, WY	open	normal	yes	R	
Bohne 1974	Lolo National Forest, MT	closed	normal	no	P	
Boyd 1970	White River Plateau, CO	closed	normal	no	R	
Burt and Gates 1959	Pecos Basin, NM	open	normal	yes	R	
Cada 1973, 1974, 1976	Upper Gallatin River, MT	open	normal	yes and no	R	
Canfield 1984	Boulder Mtns., MT	open	normal	no	F	
Casagranda 1958	Sun River WMA, MT	open	normal	no	R	
Claar 1973	Big Creek, ID	open	normal and hard	yes	R	MD
Cliff 1939	Blue Mtns, OR	open	normal	no	R	
Compton 1974	Sierra Madre, WY	closed	normal	no	F	MD
Constan 1967	Upper Gallatin River, MT	open	normal	no	P, R	MD, BHS
Cowan 1947	Jasper National Park, Alberta	open	normal	no	P, R	MD
Daneke 1980	Middle Fork Flathead R., MT	closed	hard	no	F	
Delgiudice et al. 2001	Yellowstone National Park, WY	open	normal and hard	no	F	BIS
Denio 1938a, b	National Forest, MT, ID, SD, and WA	open	normal	yes	R	MD
Eustace 1967	Wall Creek WMA, MT	open	normal	no	P	
ates 1961	Pecos Wilderness Area, NM	open	normal	yes	R	

APPENDIX A – CONTINUED

Gordon 1968	Elkhorn Mtns, MT	open	normal and hard	no	P	
Greer 1958	Upper Gallatin River, MT	open	hard	no	R	
Greer 1959	Gallatin/Yellowstone Rivers, MT	open	normal	yes	R	
Greer 1960	Sun River WMA, MT	open	normal	no	R	
Greer 1970	Yellowstone National Park, WY	open	normal and hard	yes	R	
Guse et al. 1965	Rocky Mountain National Park, CO	open	normal	no	R	
Gysel 1960	Rocky Mountain National Park, CO	open	normal	no	R	
Hansen and Reid 1975	Sangre De Cristo Mtns., CO	open	normal	no	F	MD
Holsworth 1960	Elk Island National Park, Alberta	closed	normal	no	F	MSE
Hunt 1979	Saskatchewan	closed	normal	no	R	
Jenkins and Starkey 1993	Cascade Mountains, WA	closed	normal	no	F	
Jenkins and Wright 1987	North Fork Flathead River, MT	closed	normal and hard	no	F	WTD, MSE
Kasworm et al. 1984	Sun River WMA, MT	open	normal	no	F	MD, BHS
Kirsch 1962	Little Belt Mtns., MT	open	normal	no	R	
Knight 1968	Sun River WMA, MT	open	normal	no	P	
Komberec 1976	Missouri River Breaks, MT	open	hard	no	P	MD
Kvale 1981	Phosphate Study Area, ID	closed	normal	no	P, F	MSE, MD
Lovaas 1963	Upper Gallatin River, MT	open	hard	yes	R	
MacCracken and Hansen 1981	San Juan Mountains, CO	closed	normal	no	F	MD
Mackie 1965	Missouri River Breaks, MT	open	normal	no	P, R	MD

APPENDIX A – CONTINUED

Martinka 1969	Glacier National Park, MT	open	normal	no	R	
McArthur 1977	Colockum Recreational Area, WA	open	normal	no	F	
McCorquodale 1993	Arid Lands Ecology Reserve, WA	open	normal and hard	no	F	
McReynolds 1977	Colockum Recreational Area, WA	open	normal	yes	F	
Mehus 1995	Upper Yellowstone River, MT	open	normal	no	F	MD
Morgantini and Bruns 1984	Clearwater/Blackstone Rivers, Alberta	open and closed	normal	no	F	
Morgantini and Hudson 1985	Ya Ha Tinda Ranch, Alberta	open	normal	no	F	
Morris and Schwartz 1957	National Bison Range, MT	open	normal	no	R	MD
Mower and Smith 1989	Wasatch Front, UT	closed	normal	no	F	MD
Osborn et al. 1997	Pembina Hills, ND	open	normal	yes	F, R	
Peek 1963	Gravelly Mountains, MT	open	normal	yes	R	MD
Picton 1960	Sun River WMA, MT	open	normal	no	P	
Rouse 1957	Gravelly Mountains, MT	open	normal and hard	no	P, R	
Rowland 1981	Jemez Mountains, NM	open	normal and hard	no	F	
Schallenberger 1966	Sun River WMA, MT	open	hard	no	P	WTD, MD, BHS
Short et al. 1977	Fort Bayard Watershed, NM	closed	normal	no	R	MD
Singer 1979	Glacier National Park, MT	closed	normal	no	P	MSE, MD
Singer and Norland 1994	Yellowstone National Park, WY	open	normal	no	F	MD, BIS, BHS, PA
Skovlin and Vavra 1979	Blue Mountains, OR	open	normal and hard	no	F	MD

APPENDIX A – CONTINUED

Stevens 1965	Elkhorn Mountains, MT	open	normal	no	P, R	
Sullivan 1988	Theodore Roosevelt National Park, ND	open	normal	no	F	WTD, MD, BIS
Telfer 1994	Porcupine Hills, Alberta	open	hard	no	F	MSE, MD
Telfer and Cairns 1979; Cairns 1976	Elk Island National Park, Alberta	closed	normal	no	F	WTD, BIS, MSE
Trout 1971	Lochsa River, ID	closed	normal	no	R	
Vales and Peek 1996	Yellowstone National Park, WY	open	normal and hard	no	F	
Vore 1990	Yellowstone National Park, WY	open	normal	yes	R	
Wright and Kelsey 1997	Selway Bitterroot Wilderness, ID	closed	normal	yes	P, R	WTD, MD
Wydeven and Dahlgren 1983, 1985	Wind Cave National Park, SD	open	normal	no	P, R	MD, BIS, PA

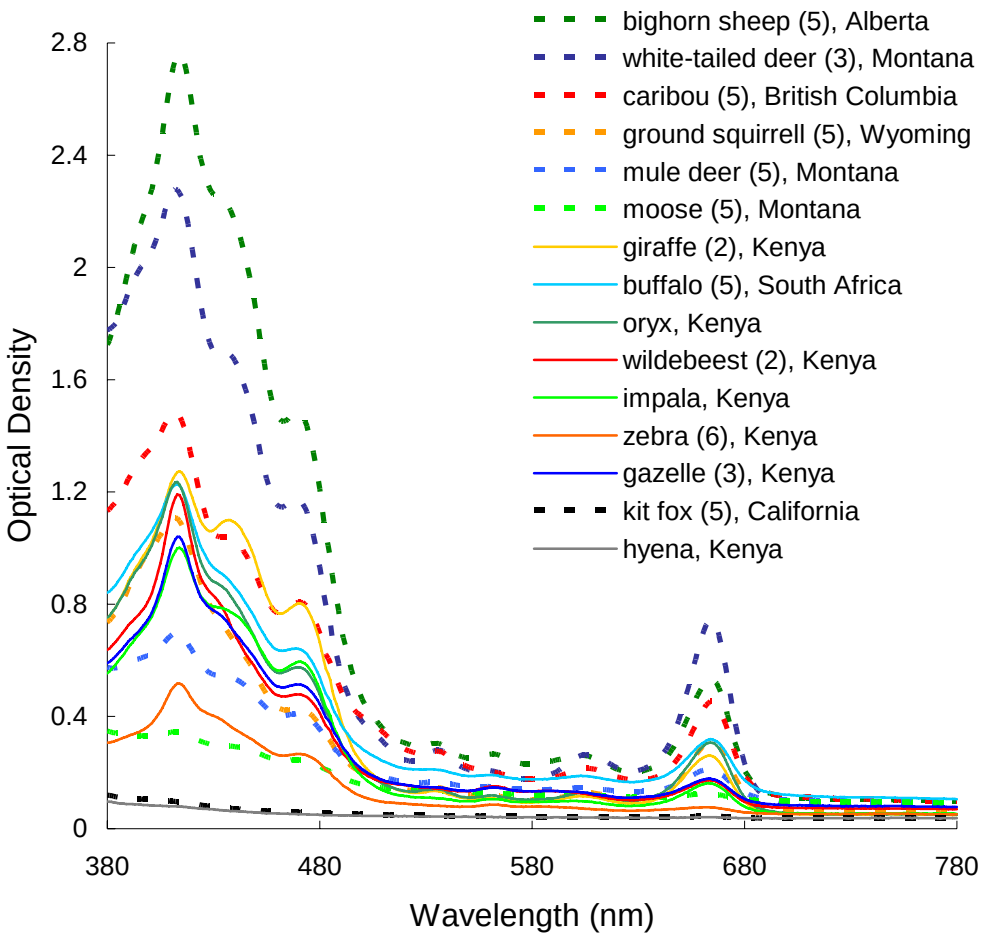
1 R = rumen diet estimate, F = fecal diet estimate, P = plant use diet estimate

2 WTD = white tailed deer, MD = mule deer, BHS = bighorn sheep, PA = pronghorn antelope, BIS = Bison, MSE = moose.

3 WMA = Wildlife Management Area

APPENDIX B

LIGHT ABSORPTION FROM 380 – 780 NM (RESOLUTION, 1 NM) FOR FECAL EXTRACTS FROM NORTH AMERICAN (DOTTED LINES) AND AFRICAN (SOLID LINES) MAMMALS.



The feces of herbivores shows broad similarities in the pattern of light absorption but with significant variation in magnitude. Light absorption is averaged for each species and parentheses indicate sample size, otherwise, n = 1. Fecal samples include white-tailed deer (*Odocoileus virginianus*) in spring and moose (*Alces alces*) and mule deer (*Odocoileus hemionus*) in winter from the upper Gallatin Canyon in southwestern Montana, USA; oryx (*Oryx gazelle*), Grant's gazelle (*Gazella granti*), wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*), zebra (*Equus quagga*), giraffe (*Giraffa camelopardalis*), and spotted hyena (*Crocuta crocuta*) in the cooler dry season in from Shompole-Olkiramatian Community Conservation Area, southern Kenya; African buffalo (*Syncerus caffer*) from Kruger National Park in South Africa; bighorn sheep (*Ovis canadensis*) in spring from the Sheep River Wildlife Sanctuary, southwestern Alberta, Canada; caribou (*Rangifer tarandus*) in winter from the Cariboo Mountains, eastern British Columbia, Canada; Uinta ground squirrel (*Spermophilus armatus*) in summer from Yellowstone National Park, northwestern Wyoming, USA, and San Joaquin kit fox (*Vulpes macrotis*) from southern California, USA.

APPENDIX C.

COMPARISON OF LINEAR, QUADRATIC, AND PIECEWISE REGRESSIONS OF ELK FECAL CHLOROPHYLL ON JULIAN DAY OVER THREE WINTER-SPRING TRANSITIONS.

Model[1]	Parameter estimates (±SE)				K	ΔAIC	r^2_a
	b_0	b_1	b_2	Break-point			
2004 fecal chlorophyll (*n* = 396)							
Linear	-0.1101 (±0.0148)	0.0039 (±0.0002)			2	698.37	0.515
Quadratic	-0.0275 (±0.0098) (P = 0.005)	-0.0024 (±0.0003)	5.1×10^{-5} ($\pm 2.0 \times 10^{-6}$)		3	249.64	0.809
Piecewise[2]	0.0308 (±0.0081)	-1.4×10^{-5} (±0.0001) (P = 0.920)	0.0129 (± 0.0004)	87.7 (±1.2)	4	0	0.887
2005 fecal chlorophyll (*n* = 382)							
Linear	-0.0080 (±0.0059) (P = 0.178)	0.0014 ($\pm 8.0 \times 10^{-5}$)			2	603.86	0.450
Quadratic	0.0014 (P = 0.727)	-0.0014 ($\pm 1.3 \times 10^{-5}$)	2.8×10^{-5} ($\pm 1.0 \times 10^{-6}$)		3	265.88	0.766
Piecewise[2]	0.0196 (±0.0029)	6.0×10^{-5} ($\pm 5.6 \times 10^{-5}$) (P = 0.265)	0.0083 (±0.0003)	90.8 (±1.0)	4	0	0.881
2006 fecal chlorophyll (*n* = 244)							
Linear	-0.0267 (±0.0117) (P = 0.023)	0.0017 (±0.0002)			2	430.68	0.341
Quadratic	0.0276 (±0.0097) (P = 0.005)	-0.0031 (±0.0004)	4.1×10^{-5} ($\pm 3.0 \times 10^{-6}$)		3	294.04	0.625
Piecewise[2]	0.0176 (±0.0050)	0.0002 ($\pm 8.2 \times 10^{-5}$) (P = 0.013)	0.0217 (±0.0010)	110.4 (±0.7)	4	0	0.888

[1] All estimates and regressions significant at $P < 0.001$ unless labeled otherwise.

[2] $b_0 + b_1 \times$ Julian day + b2 × (Julian day – breakpoint)(Julian day > breakpoint), where (Julian day > breakpoint) is a logical operator that results in 0 or 1.

APPENDIX D

COMPARISON OF LINEAR, QUADRATIC, AND PIECEWISE REGRESSIONS OF ELK FECAL NITROGEN ON JULIAN DAY OVER THREE WINTER-SPRING TRANSITIONS.

Model[1]	Parameter estimates (±SE)				K	ΔAIC	r^2_a
	b_0	b_1	b_2	Break-point			
2004 fecal nitrogen (*n* = 238)							
Linear	1.146 (±0.0148)	0.0079 (±0.0007)			2	346.99	0.378
Quadratic	1.362 (±0.0268)	0.0104 (±0.0003)	0.0002 (±7.0×10^{-6})		3	54.735	0.818
Piecewise[2]	1.433 (±0.025)	-0.0023 (±0.0006)	0.0307 (± 0.0010)	75.3 (±1.2)	4	0	0.856
2005 fecal nitrogen (*n* = 382)							
Linear	1.414 (±0.020)	0.0035 (±0.0003)			2	122.49	0.258
Quadratic	1.417 (±0.016)	-0.0028 (±0.0006)	7.2×10^{-5} (±1.0×10^{-6})		3	0	0.502
Piecewise[2]	1.453 (±0.018)	-0.0013 (±0.0007) (P = 0.082)	0.0106 (±0.0011)	47.7 (±4.3)	4	41.28	0.436
2006 fecal nitrogen (*n* = 244)							
Linear	1.332 (±0.034)	0.0043 (±0.0004)			2	41.75	0.269
Quadratic	1.430 (±0.034)	-0.0043 (±0.0013) (P = 0.001)	7.4×10^{-5} (±1.1×10^{-5})		3	0	0.387
Piecewise[2]	1.419 (±0.037)	-0.0007 (±0.0014) (P = 0.611)	0.0097 (±0.0019)	57.0 (±9.0)	4	20.33	0.342

[1] All estimates and regressions significant at $P < 0.001$ unless labeled otherwise.

[2] $b_0 + b_1 \times$ Julian day + b2 × (Julian day – breakpoint)(Julian day > breakpoint), where (Julian day > breakpoint) is a logical operator that results in 0 or 1.

APPENDIX E

COMPARISON OF LINEAR, QUADRATIC, AND PIECEWISE REGRESSIONS OF CHLOROPHYLL CONTENT OF ELK FORAGE GRASSES ON JULIAN DAY (N = 64) IN 2005.

Model[1]	Parameter estimates (±SE)				K	ΔAIC	R^2
	Intercept	b_1	b_2	Break-point			
Grass chlorophyll (n = 64)							
Linear	0.0064 (±0.0142) (P = 0.654)	0.0017 (±0.0003)			2	18.18	0.420
Quadratic	0.0109 (±0.0130) (P = 0.406)	6.0 0 × 10^{-6} (±0.0005) (P = 0.990)	0.0002 (5.0 × 10^{-6})		3	6.77	0.522
Piecewise[2]	0.0203 (±0.0124) (P = 0.110)	0.0009 (±0.0003) (P = 0.003)	0.0061 (±0.0012)	87.0 (±7.1)	4	0	0.576
Grass digestible nitrogen (n = 64)							
Linear	0.3130 (±0.0759)	0.0058 (±0.0014)			2	15.36	0.215
Quadratic	0.3380 (±0.0683)	-0.0039 (±0.0027) (P = 0.149)	0.0001 (±2.7 × 10^{-5})		3	2.19	0.371
Piecewise[2]	0.3853 (±0.0685)	0.0014 (±0.0017) (P = 0.415)	0.0295 (±0.0077)	84.4 (±7.8)	4	0	0.401

[1] All estimates and regressions significant at $P < 0.001$ unless labeled otherwise.

[2] $b_0 + b_1 \times$ Julian day + b2 × (Julian day – breakpoint)(Julian day > breakpoint), where (Julian day > breakpoint) is a logical operator that results in 0 or 1.

APPENDIX F

COMPARISON OF LINEAR, QUADRATIC, AND PIECEWISE REGRESSIONS OF NDVI AT FECAL COLLECTION POINTS ONTO JULIAN DAY OF FECAL COLLECTION OVER THREE WINTER-SPRING TRANSITIONS.

Model[1]	Parameter estimates (±SE)				K	ΔAIC	r^2_a
	b_0	b_1	b_2	Break-point			
2004 NDVI (*n* = 370)							
Linear	49.79 (±1.55)	0.480 (±0.023)			2	199.691	0.533
Quadratic	52.18 (±1.26)	-0.186 (±0.051)	0.0068 (±0.0005)		3	0.03	0.698
Piecewise[2]	55.47 (±1.23)	0.047 (±0.039) (P = 0.228)	1.285 (± 0.081)	66.3 (±2.6)	4	0	0.730
2005 NDVI (*n* = 370)							
Linear	54.81 (±1.61)	0.430 (±0.024)			2	56.02	0.459
Quadratic	56.40 (±1.51)	-0.0105 (±0.0605) (P = 0.863)	0.0045 (±0.0006)		3	0.76	0.535
Piecewise[2]	53.31 (±2.13)	-0.201 (±0.157) (P = 0.200)	0.856 (±0.161)	23.5 (±6.5)	4	0	0.540
2006 NDVI (*n* = 260)							
Linear	51.91 (±1.78)	0.359 (±0.027)			2	153.274	0.406
Quadratic	54.31 (±1.42)	-0.307 (±0.057)	0.0068 (±0.0005)		3	30.80	0.630
Piecewise[2]	57.12 (±1.38)	0.030 (±0.035) (P = 0.385)	1.591 (±0.131)	81.6 (±2.5)	4	0	0.675

[1] All estimates and regressions significant at $P < 0.001$ unless labeled otherwise.

[2] $b_0 + b_1 \times$ Julian day + b2 × (Julian day – breakpoint)(Julian day > breakpoint), where (Julian day > breakpoint) is a logical operator that results in 0 or 1.

APPENDIX G

COMPARISON OF LINEAR, QUADRATIC, AND PIECEWISE REGRESSIONS OF FECAL CHLOROPHYLL ONTO NDVI AT THE TIME OF FECAL COLLECTION AT THREE SPATIAL SCALES. ALL ESTIMATES AND REGRESSIONS SIGNIFICANT AT $P < 0.001$ UNLESS LABELED OTHERWISE.

Model[1]	Parameter estimates (±SE)				K	ΔAIC	r^2_a
	b_0	b_1	b_2	Break-point			
Fecal chlorophyll on NDVI for the pixel at collection point (n = 597)[2]							
Linear	-0.0828 (±0.0089)	0.0019 (±0.0001)			2	110.68	0.349
Quadratic	0.1294 (±0.0315)	-0.0035 (±0.0008)	2.9×10^{-5} ($\pm 4.0 \times 10^{-6}$)		3	65.31	0.397
Piecewise[3]	-0.0254 (±0.0112) (P = 0.024)	0.0010 (±0.0002)	0.0120 (± 0.0013)	131.4 (±1.4)	4	0	0.461
Fecal chlorophyll on mean NDVI for drainage (n = 1122)							
Linear	-0.2426 (±0.0112)	0.0044 (±0.0001)			2	126.77	0.514
Quadratic	0.1782 (±0.0399)	-0.0059 (±0.0010)	5.6×10^{-5} ($\pm 5.0 \times 10^{-6}$)		3	14.49	0.561
Piecewise[3]	-0.1002 (±0.0219)	0.0022 (±0.0003)	0.0073 (±0.0006)	104.8 (±2.6)	4	0	0.567
Fecal chlorophyll on mean NDVI of study area (n = 1122)							
Linear	-0.2889 (±0.0103)	0.0050 (±0.0001)			2	620.91	0.613
Quadratic	0.5990 (±0.0308)	-0.0167 (±0.0008)	0.0001 ($\pm 4.0 \times 10^{-6}$)		3	36.94	0.770
Piecewise[3]	-0.0193 (±0.0163) (P = 0.236)	0.0007 (±0.0002) (P = 0.003)	0.0130 (±0.0004)	103.5 (±1.0)	4	0	0.777

[1] All estimates and regressions significant at $P < 0.001$ unless labeled otherwise.

[2] Fecal collections from 2004 were not included because locations were recorded less precisely.

[3] $b_0 + b_1 \times$ NDVI + b2 × (NDVI – breakpoint)(NDVI > breakpoint), where (NDVI > breakpoint) is a logical operator that results in 0 or 1.