NRC Research Press

ARTICLE

Photosynthetic pigments estimate diet quality in forage and feces of elk (*Cervus elaphus*)

D. Christianson and S. Creel

Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.

Abstract: Understanding the nutritional dynamics of herbivores living in highly seasonal landscapes remains a central challenge in foraging ecology with few tools available for describing variation in selection for dormant versus growing vegetation. Here, we tested whether the concentrations of photosynthetic pigments (chlorophylls and carotenoids) in forage and feces of elk (*Cervus elaphus* L., 1785) were correlated with other commonly used indices of forage quality (digestibility, energy content, neutral detergent fiber (NDF), and nitrogen content) and diet quality (fecal nitrogen, fecal NDF, and botanical composition of the diet). Photosynthetic pigment concentrations were strongly correlated with nitrogen content, gross energy, digestibility, and NDF of elk forages, particularly in spring. Winter and spring variation in fecal pigments and fecal nitrogen was explained with nearly identical linear models estimating the effects of season, sex, and day-of-spring, although models of fecal pigments were consistently a better fit (r^2_{adjusted} = 0.379–0.904) and estimated effect sizes more precisely than models of fecal nitrogen (r^2_{adjusted} = 0.247–0.773). A positive correlation with forage digestibility, nutrient concentration, and (or) botanical composition of the diet implies fecal photosynthetic pigments may be a sensitive and informative descriptor of diet selection in free-ranging herbivores.

Key words: carotenoid, chlorophyll, diet selection, digestibility, energy, foraging behavior, nitrogen, phenology, photosynthesis, primary productivity.

Résumé : La compréhension de la dynamique nutritive des herbivores vivant dans des paysages très saisonniers demeure un des défis centraux de l'écologie de l'alimentation, peu d'outils étant disponibles pour décrire les variations du choix de plantes dormantes ou en croissances. Nous avons vérifié si les concentrations de pigments photosynthétiques (chlorophylles et caroténoïdes) dans les aliments et les fèces de cerfs élaphes (*Cervus elaphus* L., 1785) étaient corrélées à d'autres indices couramment utilisés de la qualité des aliments (digestibilité, contenu énergétique, fibres au détergent neutre (FDN) et teneur en azote) et du régime alimentaire (azote dans les fèces, FDN dans les fèces et composition botanique du régime alimentaire). Les concentrations de pigments photosynthétiques étaient fortement corrélées à la teneur en azote, à l'énergie brute, à la digestibilité et aux FDN des aliments des cerfs, en particulier au printemps. Si les variations hivernales et printanières des pigments dans les fèces et de l'azote fécal s'expliquaient par des modèles linéaires presque identiques estimant les effets de la saison, du sexe et du jour du printemps, les modèles de pigments dans les fèces étaient uniformément mieux ajustés ($r^2_{\text{ajusté}}$ = 0,379–0,904) et estimaient l'ampleur des effets plus précisément que les modèles d'azote fécal ($r^2_{\text{ajusté}}$ = 0,247–0,773). Une corrélation positive avec la digestibilité des aliments, la concentration de nutriments et (ou) la composition botanique de l'alimentation indique que les pigments photosynthétiques dans les fèces pourraient constituer un descripteur sensible et informatif du choix de l'alimentation chez les herbivores en liberté. [Traduit par la Rédaction]

Mots-clés : caroténoïde, chlorophylle, choix de l'alimentation, digestibilité, énergie, comportement d'alimentation, azote, phénologie, photosynthèse, productivité primaire.

Introduction

For many herbivores, a principle source of variation in diet quality is the photosynthetic capacity of available plant tissue, with dormant, senescent vegetation providing the least nutritional value and actively photosynthesizing tissue usually providing nutrients above maintenance levels (McNaughton 1979; Wilmshurst et al. 1995; Murray and Illius 2000; Shrader et al. 2006). Annual photosynthetic cycles are conspicuous from leaf to landscape levels and growth, survival, and reproduction in many herbivorous taxa are strongly synchronized with plant phenology (Fryxell et al. 1988; Sinclair et al. 2000; Ryan et al. 2007). Within seasons or in less seasonal landscapes, herbivores must also contend with a mosaic of forages that vary in phenology and ratio of dormant to photosynthetic tissue (McNaughton 1979; Murray and Brown 1993; Fraser and Gordon 1997; Treydte et al. 2013). The absolute or relative concentration of photosynthetic pigments (i.e., chlorophylls and carotenoids) are often used to quantify photosynthetic capacity of plants, as well as the "greenness" of forage and habitats selected by herbivores (McNaughton 1976; Misra and Misra 1981; Treydte et al. 2013). This approach includes the more recent and rapidly growing use of indices of primary productivity derived from satellite imagery to estimate habitat quality for herbivores (Pettorelli et al. 2005).

Perhaps the most frequently used and direct measures of free-ranging herbivore diet selection involve the physicochemical or botanical composition of herbivore feces (Wofford et al. 1985; Wehausen 1995). Feces provides a readily available, noninvasively collected sample largely composed of the plants and compounds actually consumed by a free-ranging population (Wofford et al.

Received 22 June 2014. Accepted 3 November 2014.

D. Christianson. School of Natural Resources and the Environment, University of Arizona, Biological Sciences East 325, Tucson, AZ 85721, USA.
S. Creel. Department of Ecology, Montana State University, 310 Lewis Hall, Bozeman, MT 59717, USA.
Corresponding author: David Christianson (e-mail: dchristianson@email.arizona.edu).

Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.

1985; Wehausen 1995). Although many fecal metrics exist to describe diet selection in herbivores, the use of photosynthetic pigments in feces to describe diet selection or diet quality remains entirely unexplored (but see Christianson and Creel 2009). This is surprising given the longstanding use and inferential power of qualitative descriptors of photosynthetic capacity (or greenness) to index herbivore forage, habitat, or landscape quality for herbivores (McNaughton 1976), an approach that continues to generate important inferences into herbivore foraging behavior and ecosystem functioning (Treydte et al. 2013). This mismatch between dietary and environmental descriptors of forage quality is particularly surprising given that photosynthetic pigments were found to be indigestible and easily extracted from feces over 60 years ago (Reid et al. 1950; Smart et al. 1954). Photosynthetic pigments in feces were extensively studied for use as a marker for estimating dry matter digestiblility of forages in feeding trials with domestic ruminants and waterfowl (Reid et al. 1950; Deijs and Bosman 1955; Davis et al. 1968; Drent et al. 1979; Lowry and Schlink 1995) and as an index of algae consumption in aquatic invertebrates (Szymczak-Żyla et al. 2006). Consequently, although digestive kinetics and fecal recovery of pigments in herbivores are relatively well-understood, correlations between fecal pigment concentration and nutritional characteristics of the diet have never been explored in a free-ranging herbivore. It has been shown that fecal chlorophyll in herbivores increases as the availability of photosynthetic plant tissue on the landscape increases (Lowry and Schlink 1995; Christianson and Creel 2009), so it is logical to assume fecal photosynthetic pigments may provide a meaningful measure of consumption of green, photosynthetic tissue, which may correlate with more specific measures of diet quality in terrestrial herbivores. Furthermore, it is widely accepted that a primary effect of climate change on herbivores will operate through shifts in plant phenology, including the onset of the growing season and growing rates of forage plants (Forchhammer and Post 2004; Berteaux et al. 2006; Christianson et al. 2013). A metric that is sensitive to the photosynthetic pigment concentration of plants consumed by herbivores may be useful for detecting higher trophic level effects of phenological shifts due to climate change.

Currently, fecal nitrogen is the most commonly measured fecal component in free-ranging herbivores for describing variation in diet selection and diet quality (Leslie et al. 2008). In wild herbivores, variation in fecal nitrogen correlates with temporal patterns of plant phenology (Leslie and Starkey 1985; Irwin et al. 1993; Massey et al. 1994) and has been used to identify drivers of variation in diet quality at relatively coarse scales, for example, differences in diet quality across seasons in bighorn sheep (*Ovis canadensis* Shaw, 1804) (Irwin et al. 1993). Increasingly, fecal nitrogen is being applied to questions at finer scales, including interspecific competition (Lin et al. 2011), habitat selection (Ryan et al. 2012), and population dynamics (Blanchard et al. 2003), and has been subsequently measured in over 40 mammalian taxa (reviewed by Leslie et al. 2008) demonstrating the demand for finely resolved, noninvasive metrics of diet selection. Thus, to evaluate whether a new metric is practical for addressing such questions, a meaningful benchmark would be its ability to generate similar or novel inferences into herbivore foraging ecology compared with fecal nitrogen. We hypothesized that photosynthetic pigment concentrations were strongly correlated with other physicochemical properties of forage and feces in a free-ranging wild herbivore, the elk (*Cervus elaphus* L., 1785). We predicted that the direction of these correlations was positive with respect to physicochemical metrics of diet quality (e.g., nitrogen content, energy content, digestibility) so that high pigment concentrations correspond with high quality. We then tested whether fecal pigments generated similar or novel conclusions, compared with fecal nitrogen, regarding key factors that influenced the foraging ecology of a large terrestrial herbivore.

Materials and methods

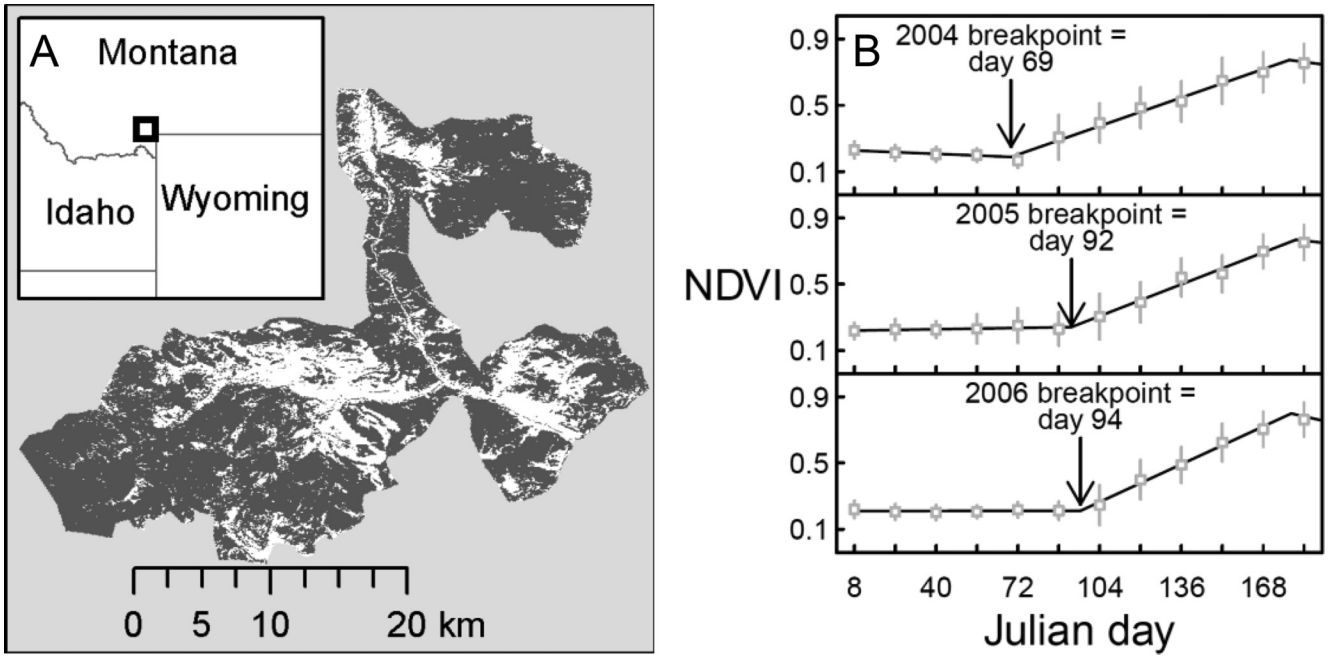
Study area and sampling design

We collected feces and forage samples from the winter and spring range of elk in the Upper Gallatin river drainage of southwestern Montana (Fig. 1A). This herd (approximately 1100 animals from aerial counts between 2003 and 2005) occupied high elevation (>2400 m), subalpine and alpine habitats in the Gallatin and Madison mountain ranges in summer and fall and lower (<2200 m) mountain forests and foothills along the Gallatin River in winter and spring. At this latitude and elevation, the bulk of net annual primary production occurs between April and October (Christianson et al. 2013), and aboveground herbaceous biomass is largely dormant and senescent outside the growing season. Elk in this population, like many herbivores, faced significant nutritional restriction in winter and significant variation in the quality of forage items available (Christianson and Creel 2010), particularly in spring when new green growth emerges among the senescent vegetation of the prior growing season (Christianson and Creel 2009). Previous research found significant differences in diet composition and nutritional condition in response to predation risk from wolves (*Canis lupus* L, 1758), habitat type, snowpack, time of year, and weather (Creel and Winnie 2005; Creel et al. 2005; Winnie and Creel 2007; Christianson and Creel 2008, 2010); therefore, this population is suitable for comparing the ability of different metrics to explain biologically significant variation in diet selection in a free-ranging herbivore.

The study area, elk population, and sampling methods have been described in detail previously (Christianson and Creel 2008, 2009, 2010; Creel and Christianson 2009). Briefly, from December to May in 2004, 2005, and 2006, we located elk groups in three tributaries of the Gallatin River approximately every 14 days, recorded the group composition (adult female, juvenile, adult male, yearling male), and selected 3–10 fresh (<24 h) fecal samples (30 mL each) from individual piles of fecal pellets. For most fecal samples (59%), the sex composition of the group being sampled could be confirmed visually just prior to collection. Most (92%) elk during this study were in groups >90% or <10% adult male (based on classification of 13 104 elk in 1 138 groups), with most males found in a single drainage with few females and juveniles. Because of this strong social and spatial sexual segregation (Winnie and Creel 2007; Christianson and Creel 2008), as well as because fecal pellets differed significantly in size between adult males and females, allowing for secondary confirmation of sex (Creel and Christianson 2009), all fecal samples from unknown groups could be dichotomized as coming from groups composed primarily of adult males or groups composed primarily of adult females and juveniles (Christianson and Creel 2008; Creel and Christianson 2009). We refer to the latter group simply as "females" because 82% of elk in nonmale-biased groups were adult females (based on classification of 13 104 elk in 1 138 groups).

Throughout each winter and spring, we collected vegetation samples from elk foraging sites (determined by direct observation of foraging elk) representing six major forage categories that could be unambiguously identified microscopically in fecal samples (see below): graminoids (n = 121 samples), conifer needles (n = 31), sagebrush (n = 28), woody browse stems (n = 22), willow and aspen stems (n = 12), forbs (n = 4), and two less important species that were easily identifiable microscopically: spiny phlox (*Phlox hoodii* Richardson) (n = 3) and creeping barberry (*Mahonia repens* (Lindl.) G. Don) (n = 3). Vegetation samples also included "grazing" samples (n = 25) containing mixed graminoids and forbs from elk foraging sites, which we collected using grab-and-clip sampling to simulate foraging in patches located immediately adjacent (offset by <1 m) to elk foraging patches that we identified from direct observation and from tracks in the snow or soil. Hand-sorting revealed these grazing samples were 93.8% ± 13.7% (mean ± SD) graminoids, so we refer to these as "graminoids" for simplicity.

Fig. 1. (A) Winter–spring range of elk (*Cervus elaphus*) along three drainages of the Gallatin River in in southwestern Montana, USA, is composed of a matrix of (dark shading) forested and (unshaded) open grasslands and shrublands (30 m resolution). (B) Time series of satellite-derived Normalized Difference Vegetation Index (NDVI) values from open habitats (16-day frequency, 250 m resolution) used to identify the transition between winter and spring in the first half of each of the 3 years of this study (2004–2006). Box and whiskers are mean and SD of NDVI values at each 16-day sampling period by the MODIS satellite. Piecewise regression estimated dates when trend in NDVI significantly changed (breakpoints), indicating snowmelt and the onset of spring growth.



All vegetation samples were assayed individually for in vitro dry matter digestibility (%), gross energy (calories per gram, where 1 calorie = 4.1858 J at 15 °C), nitrogen (% dry matter), and neutral detergent fiber (NDF; % dry matter) at an independent, commercial laboratory. Previous research found elk in this population were primarily grazers, with microhistological diet estimates reporting 72.8% ± 19.0% (mean ± SD) graminoids in the diet (Christianson and Creel 2008), the remainder being browsed stems of deciduous and evergreen trees and shrubs. Thus, we focus our analysis primarily on the nutritional properties of graminoids (but an analysis considering all forage types was also considered and is reported in the Results below).

Fecal samples were assayed for nitrogen content (% dry matter) at an independent laboratory. Due to cost constraints, only a subset from a single year, 2004 (n = 218), was also assayed for NDF (% dry matter). Botanical composition of elk diets was determined for each fecal sample using microhistology to assign epidermal plant fragments in elk feces to one of the eight forage categories described above for vegetation sampling (Sparks and Malechek 1968), which we then pooled into either a "browse" or graminoid category. Forbs were consistently <5% of the diet and were included with the graminoid portion of the diet (Christianson and Creel 2008). Because the grass and browse diet categories sum to one and statistical analysis on both categories would be redundant, we focus our analysis on the browse proportion of the diet.

We measured photosynthetic pigments (chlorophylls and carotenoids) in each fecal and forage sample following standard procedures described in detail previously (Christianson and Creel 2009). Briefly, we extracted pigments by boiling a known dry mass of feces or forage (approximately 0.2 g) in 10 mL ethanol and reconstituting pigments in 1 mL methanol after a drying stage. Extracts were diluted 31-fold in methanol and scanned immediately on a 96-well microplate spectrophotometer. Optical densities at 470 nm (peak absorption specific to carotenoids) and 666 nm (peak absorption specific to chlorophylls) were recorded, as well as at 750 nm as a correction for turbidity (Lichtenthaler 1987). Several equations exist for deriving absolute pigment concentrations from the light absorption properties of solutions (Lichtenthaler 1987). No such equations have been developed for fecal extracts in methanol; therefore, we present pigment concentrations as optical densities/0.2 g sample.

Data analysis

Variation in foraging behavior or nutrition is commonly compared across seasons or across ecological circumstances within a season. To compare the behavior of dietary metrics across and within season, we demarcated two seasons (winter and spring) in each year of our study using Normalized Difference Vegetation Index (NDVI) values (16-day frequency, 250 m resolution) from all grassland and shrubland habitats on the study site (Fig. 1A). Applying the "segmented" package in R version 2.15.12 (R Development Core Team 2012), we used piecewise regression (broken-stick or breakpoint regression) of NDVI on Julian day to model temporal trends in NDVI and identify a breakpoint in the annual trend (Fig. 1B). The breakpoint identified the date each year when the trend in NDVI transitioned from stable (winter) to increasing (spring) indicating the onset of snowmelt and the growing season (Christianson et al. 2013). The estimated date of these breakpoints in each year was estimated with high precision (95% confidence intervals (CIs) of ±0.8–1.1 days), and we used breakpoints to classify fecal and forage samples to season and to define day zero in each year for modelling the day-of-spring effect of growing-season progression on elk diets (see below).

To compare the behavior of dietary metrics across ecological circumstances, besides season or day-of-spring, many other factors that affect diet can be found in the literature. Previous research from this population revealed male and female elk differ markedly in many aspects of foraging ecology. Male and female elk differ in activity patterns, antipredator responses, nutritional constraints, habitat selection, and diet selection (Creel and Winnie 2005; Creel et al. 2005; Winnie and Creel 2007; Christianson and

Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.

Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.

Creel 2008, 2010). For the purpose of this analysis, it should be meaningful to evaluate the characteristics of photosynthetic pigments and other dietary metrics as they vary with sex. Our interest here is not to explain differences between the sexes, only to evaluate and to compare the ability of fecal metrics to detect sex-specific differences in foraging ecology that were conspicuous using behavioral and physiological approaches.

Variance of photosynthetic pigments and physicochemical properties of elk forage and feces

For statistical comparison of metrics, we focused primarily on comparison of chlorophyll and nitrogen, because chlorophyll is the primary photosynthetic pigment in terrestrial plants (Rabinowitch and Govindjee 1969) and fecal nitrogen is a popular noninvasive metric used in terrestrial herbivores to describe diet quality (Leslie et al. 2008), but we report results from other metrics when our analysis suggested contrasting patterns or novel inferences with those metrics. We converted all metrics to their z score (i.e., subtracting the mean and dividing by the SD) so we could make pairwise comparison of the variance (s^2) in each metric between seasons (winter vs. spring) and between metrics within a season by estimating the 95% CI of variance ratios with an F test for unity in the variance ratio. We also estimated all pairwise correlations (r) between plant pigments (optical densities of chlorophyll and carotenoids), nitrogen (% dry matter), gross energy (kilocalories per gram), in vitro digestibility (% dry matter), and NDF (% dry matter). Significant correlations would indicate potential for interpreting concentrations of photosynthetic pigments as an index of diet quality.

Regression of fecal metrics on sex, season, and day-of-spring

Finally, we used least-squares regression to fit linear models for fecal pigments, fecal nitrogen, and fecal NDF as a function of sex and season using all fecal samples and directly compared coefficient estimates (mean ± SE) and mean responses between metrics. Within spring samples, we also fit linear models with sex and day-of-spring as explanatory variables because in spring we expected diet quality to change rapidly as snow melted and growing seasons progressed (Fig. 1B). We evaluated model fit using adjusted correlation coefficients (r^2_{adjusted}). Again our purpose for fitting these models is not to explain variation in fecal chlorophyll and fecal nitrogen per se, but to compare the amount of explained variation in each metric when fitted to the same model structure, as well as for comparing the magnitude, direction, and precision of the coefficient estimates for factors established, a priori, as being important for elk nutrition and diet selection in this population (Christianson and Creel 2008, 2009, 2010; Creel and Christianson 2009). Because these models were fitted using z scores for response variables and derived from the same set of samples, comparing coefficient estimates and mean responses allows for a robust comparison of the ability of each metric to generate inference into elk diet quality. All statistical estimates, tests, and models were conducted with the R statistical programming language (R Development Core Team 2012).

Results

Elk forage: photosynthetic pigments and physicochemical properties

Estimated variance in both nitrogen and chlorophyll contents of graminoids was approximately an order of magnitude higher in spring than in winter (95% CI of spring:winter variance ratio—chlorophyll: 5.3–23.6; nitrogen: 3.7–17.9). The ratio of the variances in chlorophyll to nitrogen in graminoids within a season were closer to unity (95% CI of chlorophyll:nitrogen variance ratio—winter: 1.1–2.3; spring: 0.3–2.5). In spring, pigments showed the strongest correlations across all physicochemical properties of graminoids: nitrogen, NDF, gross energy, and digestibility (Table 1). Graminoid nitrogen and NDF showed no relationship

Table 1. Correlations (r) among photosynthetic pigments and physicochemical components (NDF, neutral detergent fiber; IVDMD, in vitro dry matter digestibility) of graminoid samples commonly consumed by elk (*Cervus elaphus*) in winter and spring and all forage types across both seasons.

Forage component	Chlorophyll	Carotenoids	Nitrogen	NDF	Gross energy
Winter graminoids					
Carotenoids	**0.969 ($F_{[1,103]}$ = 1595)**				
Nitrogen	**0.722 ($F_{[1,103]}$ = 112.2)**	**0.726 ($F_{[1,103]}$ = 114.7)**			
NDF	**−0.694 ($F_{[1,103]}$ = 95.74)**	**−0.706 ($F_{[1,103]}$ = 102.5)**	**−0.642 ($F_{[1,106]}$ = 74.28)**		
Gross energy	−0.04 ($F_{[1,102]}$ = 0.17)	0.014 ($F_{[1,102]}$ = 0.02)	−0.070 ($F_{[1,105]}$ = 0.51)	0.214 ($F_{[1,105]}$ = 5.02)	
IVDMD	**0.231 ($F_{[1,103]}$ = 5.79)**	**0.269 ($F_{[1,103]}$ = 8.03)**	**0.355 ($F_{[1,106]}$ = 15.25)**	**−0.105 ($F_{[1,106]}$ = 1.19)**	0.150 ($F_{[1,105]}$ = 2.41)
Spring grazing patches					
Carotenoids	**0.988 ($F_{[1,16]}$ = 657.5)**				
Nitrogen	**0.974 ($F_{[1,14]}$ = 257.9)**	**0.955 ($F_{[1,14]}$ = 143.9)**			
NDF	**−0.85 ($F_{[1,13]}$ = 33.76)**	**−0.841 ($F_{[1,13]}$ = 31.38)**	**−0.762 ($F_{[1,18]}$ = 24.95)**		
Gross energy	**0.608 ($F_{[1,12]}$ = 7.05)**	**0.622 ($F_{[1,12]}$ = 7.57)**	0.282 ($F_{[1,17]}$ = 1.47)	0.013 ($F_{[1,17]}$ = 0.00)	
IVDMD	**0.876 ($F_{[1,14]}$ = 46.03)**	**0.863 ($F_{[1,14]}$ = 40.91)**	**0.768 ($F_{[1,19]}$ = 27.37)**	**−0.836 ($F_{[1,18]}$ = 41.84)**	0.134 ($F_{[1,17]}$ = 0.31)
All forages, winter and spring					
Carotenoids	**0.908 ($F_{[1,199]}$ = 934.4)**				
Nitrogen	**0.596 ($F_{[1,197]}$ = 108.6)**	**0.535 ($F_{[1,197]}$ = 78.96)**			
NDF	**−0.486 ($F_{[1,195]}$ = 60.14)**	**−0.625 ($F_{[1,195]}$ = 124.8)**	**−0.403 ($F_{[1,234]}$ = 45.45)**		
Gross energy	**0.307 ($F_{[1,193]}$ = 20.11)**	**0.506 ($F_{[1,193]}$ = 66.34)**	0.126 ($F_{[1,228]}$ = 3.69)	**−0.704 ($F_{[1,228]}$ = 223.6)**	
IVDMD	**0.311 ($F_{[1,197]}$ = 21.03)**	**0.312 ($F_{[1,197]}$ = 21.26)**	**0.456 ($F_{[1,237]}$ = 62.27)**	**−0.384 ($F_{[1,234]}$ = 40.51)**	**0.155 ($F_{[1,228]}$ = 5.61)**

Note: Significant ($P < 0.05$) correlations are shown in boldface type.

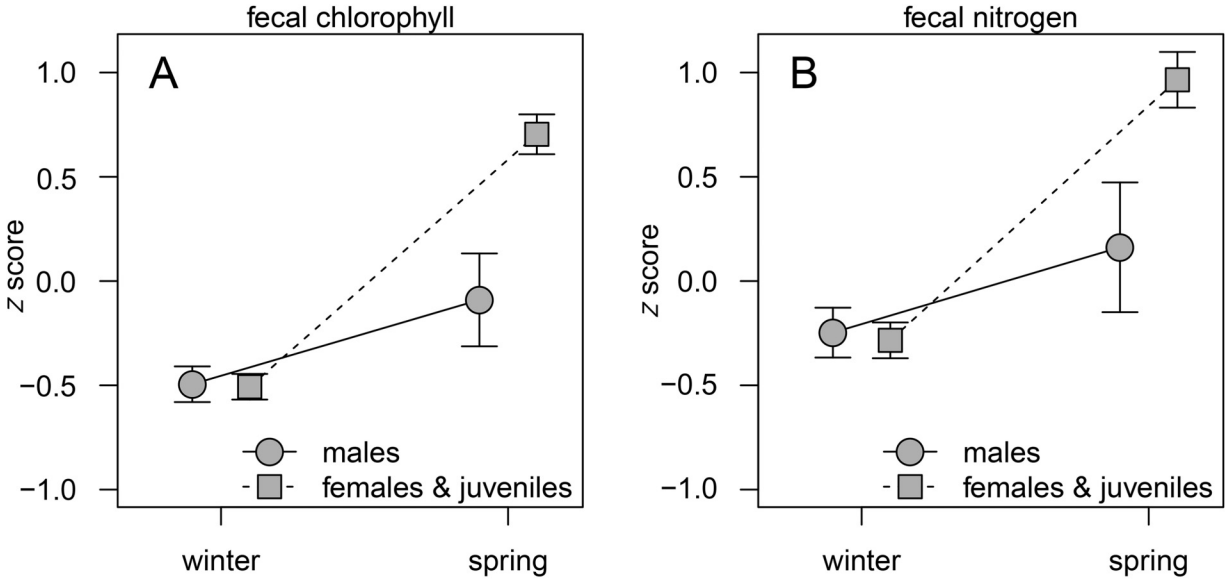
Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.

Table 2. Correlations (*r*) among photosynthetic pigments and physicochemical components (NDF, neutral detergent fiber) of fecal samples from elk (*Cervus elaphus*) in winter, spring, and both seasons.

Fecal component	Chlorophyll	Carotenoids	Nitrogen	NDF
Winter fecal samples				
Carotenoids	**0.762 ($F_{[1,638]}$ = 885.7)**			
Nitrogen	**0.378 ($F_{[1,595]}$ = 98.92)**	**0.159 ($F_{[1,595]}$ = 15.43)**		
NDF	−0.104 ($F_{[1,143]}$ = 1.57)	0.117 ($F_{[1,143]}$ = 1.99)	**−0.290 ($F_{[1,143]}$ = 13.18)**	
% browse in diet	**0.148 ($F_{[1,618]}$ = 13.89)**	**0.464 ($F_{[1,618]}$ = 169.49)**	**−0.114 ($F_{[1,583]}$ = 7.72)**	**0.472 ($F_{[1,138]}$ = 39.46)**
Spring fecal samples				
Carotenoids	**0.967 ($F_{[1,477]}$ = 6898)**			
Nitrogen	**0.893 ($F_{[1,191]}$ = 751.2)**	**0.920 ($F_{[1,191]}$ = 1054)**		
NDF	**−0.316 ($F_{[1,71]}$ = 7.88)**	**−0.291 ($F_{[1,71]}$ = 6.57)**	**−0.290 ($F_{[1,71]}$ = 6.52)**	
% browse in diet	**−0.296 ($F_{[1,354]}$ = 34.02)**	**−0.288 ($F_{[1,354]}$ = 32.09)**	**−0.285 ($F_{[1,189]}$ = 16.68)**	−0.026 ($F_{[1,70]}$ = 0.05)
All fecal samples				
Carotenoids	**0.977 ($F_{[1,1117]}$ = 23230)**			
Nitrogen	**0.817 ($F_{[1,788]}$ = 1579)**	**0.822 ($F_{[1,788]}$ = 1637)**		
NDF	**−0.224 ($F_{[1,216]}$ = 11.43)**	**−0.191 ($F_{[1,216]}$ = 8.21)**	**−0.279 ($F_{[1,216]}$ = 18.21)**	
% browse in diet	**−0.337 ($F_{[1,974]}$ = 125.2)**	**−0.313 ($F_{[1,974]}$ = 106.2)**	**−0.283 ($F_{[1,774]}$ = 67.64)**	**0.341 ($F_{[1,210]}$ = 27.59)**

Note: Significant ($P < 0.05$) correlations are shown in boldface type.

Fig. 2. Mean (±95% confidence interval (CI)) responses estimated with least-squares linear regression of (A) fecal chlorophyll and (B) fecal nitrogen on sex and season in fecal samples from elk (*Cervus elaphus*) collected in winter and spring (n = 790). Similar effect sizes were estimated in both linear models, but more precise coefficient estimates in the linear model for chlorophyll resulted in tighter CIs.



with gross energy. In winter graminoids, photosynthetic pigments showed strong correlations with nitrogen, and the strongest correlations with NDF, but weak relationships with graminoid digestibility and no relationship with energy content (Table 1). All correlations were generally weaker when examined across all seasons and forage types (graminoids, forbs, and browse), but significant relationships persisted between pigments and all physicochemical traits of forages in this broader analysis (Table 1).

Elk feces: photosynthetic pigments and physicochemical properties

In elk feces, the estimated variance in chlorophyll was much greater in spring than in winter (95% CI of spring:winter variance ratio: 180.9–286.9), while variation in fecal nitrogen in spring compared with winter was more equivalent (5.2–8.2). Consequently, the estimated variance in fecal nitrogen was greater than fecal chlorophyll in winter (95% CI of nitrogen:chlorophyll variance ratio: 38.9–53.6), but variances were similar between metrics in spring (1.1–1.8). Although fecal photosynthetic pigments were largely invariant in winter, weak but significant correlations with fecal nitrogen were detected and this correlation became very strong in spring (Table 2). There was a positive correlation between fecal pigments and % browse in the diet in winter that became a negative correlation in spring (Table 2). Fecal NDF was generally only weakly correlated with other fecal metrics and showed the strongest correlation with % browse in the diet in winter (Table 2).

Linear regression of fecal metrics on sex and season

Linear regression of the effects of sex, season, and their interaction on fecal nitrogen or fecal chlorophyll revealed seemingly identical models with similar mean responses (Figs. 2A, 2B). These simple models generally fit the data poorly, but serve to illustrate that fecal chlorophyll and fecal nitrogen could detect similar differences in diet arising from the previously identified as being important effects of sex, season, and an interaction between sex and season (Christianson and Creel 2008, 2009, 2010). Fecal chlorophyll was a better fit ($r^2_{adjusted}$ = 0.379) with this model structure than fecal nitrogen ($r^2_{adjusted}$ = 0.247) and coefficient estimates from the model of fecal chlorophyll were approximately one-third more precise than coefficient estimates from the model of fecal nitrogen (Table 3). Linear regression of fecal carotenoids produced

Table 3. Coefficient estimates (mean ± SE) and *P* values (in parentheses) from linear regression fecal pigments, fecal nitrogen, and fecal neutral detergent fiber (NDF) on sex and season (winter or spring) in all fecal samples from elk (*Cervus elaphus*).

Response variable	Intercept	Sex*	Season†	Sex × season	df
Fecal chlorophyll	−0.495±0.045 (<0.001)	−0.012±0.054 (0.822)	0.404±0.125 (<0.001)	0.806±0.135 (<0.001)	786
Fecal carotenoids	−0.493±0.043 (<0.001)	−0.054±0.054 (0.311)	0.450±0.120 (<0.001)	0.777±0.137 (<0.001)	786
Fecal nitrogen	−0.247±0.061 (<0.001)	−0.037±0.075 (0.622)	0.409±0.170 (0.016)	0.840±0.188 (<0.001)	786
Fecal neutral detergent fiber‡	0.114±0.130 (0.381)	−0.077±0.169 (0.650)	−0.650±0.466 (0.165)	0.505±0.493 (0.307)	214

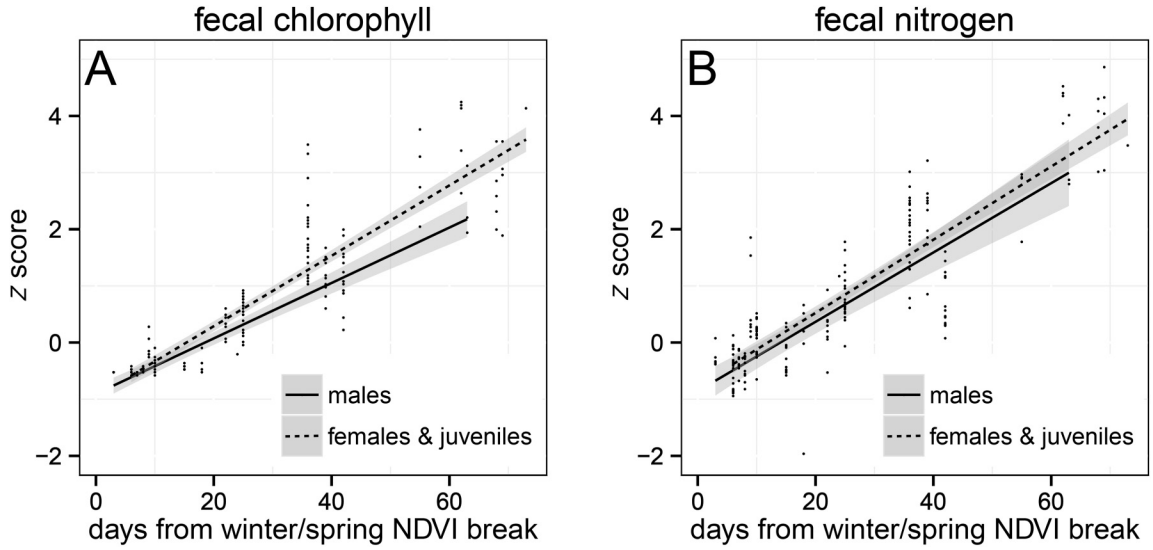
*Adult male is the reference category.
†Winter is the reference season.
‡Coefficient estimates from this model cannot be directly compared with above models because a smaller subset of observations was used to fit this model.

Table 4. Coefficient estimates (mean ± SE) and *P* values (in parentheses) from linear regression of fecal pigments, fecal nitrogen, and fecal neutral detergent fiber (NDF) on sex and day-of-spring (day) in fecal samples from elk (*Cervus elaphus*) collected in spring.

Response variable	Intercept	Sex*	Day†	Sex × day	df
Fecal chlorophyll	−0.906±0.128 (<0.001)	−0.048±0.145 (0.739)	0.049±0.005 (<0.001)	0.013±0.006 (0.022)	189
Fecal carotenoids	−0.955±0.098 (<0.001)	−0.042±0.111 (0.704)	0.054±0.004 (<0.001)	0.008±0.004 (0.064)	189
Fecal nitrogen	−0.858±0.177 (<0.001)	0.026±0.200 (0.895)	0.061±0.007 (<0.001)	0.006±0.008 (0.438)	189
Fecal NDF‡	−7.279±7.379 (0.327)	7.454±7.382 (0.316)	1.088±1.188 (0.363)	−1.101±1.188 (0.357)	69

*Adult male is the reference category.
†Days from the estimated breakpoint in the piecewise regression of Normalized Difference Vegetation Index (NDVI) values in Julian day, indicating the onset of green-up.
‡Coefficient estimates from this model cannot be directly compared with above models because a smaller subset of observations was used to fit this model.

Fig. 3. Least-squares linear regression (±95% confidence band) of (A) fecal chlorophyll and (B) fecal nitrogen on sex and day-of-spring in fecal samples from elk (*Cervus elaphus*) collected in spring (n = 193). Similar effect sizes were estimate in both linear models, but more precise coefficient estimates in the linear model for fecal chlorophyll resulted in tighter confidence bands and the detection of a sex × day-of-spring interaction not detected in fecal nitrogen.



model estimates that were very similar to fecal chlorophyll with a similar model fit ($r^2_{adjusted}$ = 0.386).

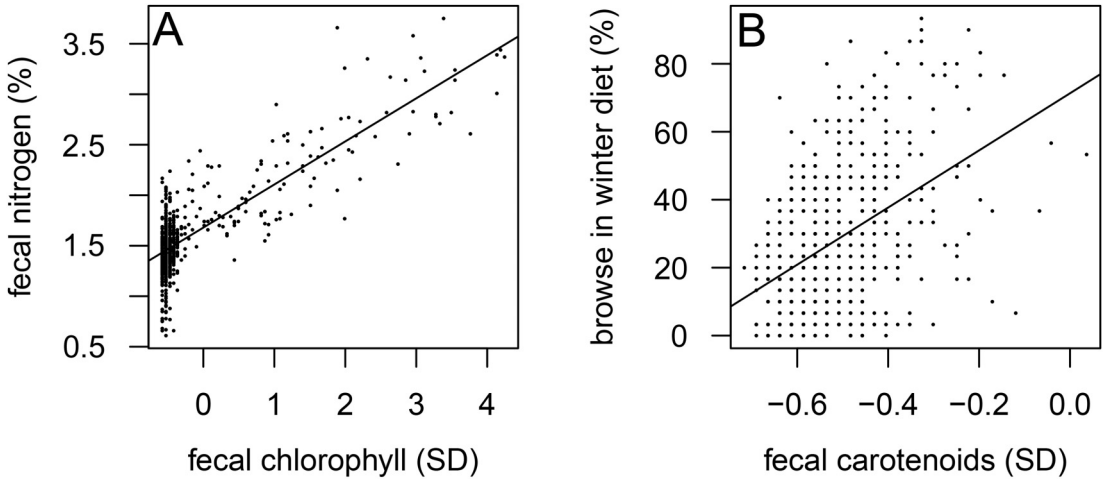
Linear regression of fecal metrics on sex and day-of-spring

In spring, linear regressions of fecal chlorophyll and fecal nitrogen produced nearly identical parameter estimates of the effects of sex and day-of-spring (i.e., days from the year-specific NDVI breakpoint). Similar effects of day-of-spring (positive; Table 4) and sex (no effect; Table 4) were seen regardless of whether fecal nitrogen or fecal pigments were the regressand (Figs. 3A, 3B). However, both fecal pigments suggested an interaction between sex and day-of-spring that was not detected in fecal nitrogen (Table 4). Coefficient estimates were more precise in models of fecal pigments compared with the model for fecal nitrogen (Table 4). Again, the model fit for fecal chlorophyll was better ($r^2_{adjusted}$ = 0.845) than the model fit for fecal nitrogen ($r^2_{adjusted}$ = 0.773). Coefficient estimates from the liner regression of fecal carotenoids were very similar to coefficients for fecal chlorophyll, but model fit was very high ($r^2_{adjusted}$ = 0.906). Because an identical set of samples were used to fit these models and the fecal pigments and fecal nitrogen were converted to *z* scores (centered and scaled by their respective means and SD) before fitting these models, these comparisons revealed important differences in the efficiency with which each metric might generate inference into elk foraging ecology.

Due to cost constraints, fecal NDF was measured within a smaller subset of samples from 1 year (winter: n = 146; spring:

Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.

Fig. 4. Post hoc estimation of linear relationships using least-squares regression between (A) fecal nitrogen (% dry matter) and fecal chlorophyll from elk (*Cervus elaphus*) and (B) the percentage of the winter diet that was browsed by elk (estimated microhistologically) and fecal carotenoids.



n = 73). Linear regressions with sex and season (with all samples) or sex and day-of-spring (within spring samples) revealed no significant relationships with fecal NDF (Tables 3, 4) and no variation in NDF could be explained by either of these models ($r^2_{adjusted} \leq 0.031$). As a post hoc analysis, we refit models for fecal pigments and fecal nitrogen to this smaller subset of data and found only a significant effect of the interaction between sex and season, but substantially greater variation in fecal pigments and nitrogen was still explained, despite the smaller sample size (sex × season model: $r^2_{adjusted}$ = 0.153–0.176; sex × day-of-spring models: $r^2_{adjusted}$ = 0.742–0.904).

Discussion

Very strong correlations were detected between photosynthetic pigments and composition of elk forage and feces in spring when the landscape (Fig. 1B) and vegetation (see Results) showed high variation in productivity and quality. Strong correlations suggest broad scope for interpreting fecal photosynthetic pigments as an index of variation in diet selection and diet quality. However, the weakest relationships between any fecal metric and important explanatory covariates (e.g., sex) were seen in winter even though sex is known to influence diet selection and nutritional plane in this population (Christianson and Creel 2008, 2010). Thus, fecal metrics may be limited in their ability to describe biologically significant variation in diet quality during periods when nutrition may have strong effects on population dynamics (Delgiudice et al. 1991; Christianson and Creel 2010). We suggest additional research with photosynthetic pigments is needed to verify the strong relationship between pigment concentration and forage quality and to confirm whether variation in fecal pigment concentrations can be used to explain variation in animal nutritional condition.

Chlorophyll and carotenoids were strongly correlated with each other in both feces and forage (Tables 1, 2), but some differences between the two pigments were apparent. These differences were at least in part an artifact of a preponderance of zero values for fecal chlorophyll in winter (77 samples or 15.7%) limiting the scope for inference from fecal chlorophyll in winter—fecal carotenoid and nitrogen estimates in winter were always >0. We used a dilution of fecal extracts that was optimized for precision in high chlorophyll samples, restricting optical densities to <1, the threshold beyond which the assumptions of Beer's Law for measuring concentrations from optical interference are less robust. This likely resulted in over dilution of low chlorophyll winter samples beyond the range of sensitivity of our spectrophotometer. Sensitivity to carotenoids was maintained because carotenoids are more detectable than chlorophylls with spectrophotometry of solutions, even though carotenoids normally persist at lower concentrations in vegetation (Lichtenthaler 1987). Consequently, we caution against the interpretation that fecal chlorophyll and fecal carotenoids described unique and different relationships with the winter diet—for elk, these pigments would likely produce similar patterns and relationships if chlorophyll dilution had remained within the range of sensitivity. In herbivores that consume dicot leaves, flowers, or fruits regularly (i.e., high carotenoid tissues), these two pigments may describe unique aspects of diet composition. Additional research is required to examine whether variability in relative concentration of each photosynthetic pigments corresponds with variability in diet selection.

Fecal pigments showed important relationships with forage quality and diet composition for interpreting diet quality, e.g., post hoc regression revealed a 1 SD increase in fecal chlorophyll corresponded with a 1.00 SD (±0.03 SE) increase in fecal nitrogen, i.e., a 0.41% increase in absolute fecal nitrogen concentration (Fig. 4A). We detected relationships unique to fecal pigments, not seen with fecal nitrogen, e.g., a 1 SD in winter fecal carotenoids corresponded to the equivalent of a 4.14 SD (±0.32 SE) increase in the proportion of browse in the diet, i.e., an 84% increase in the absolute proportion of woody browse in the winter diet (Fig. 4B). Compared with fecal nitrogen, more variation was explained and more precise coefficient estimates were seen in pigment models known, a priori, to capture much of the variation in elk foraging ecology (Tables 3, 4). This difference in precision implies fecal pigments may detect important patterns in herbivore foraging ecology that fecal nitrogen cannot, depending on the size of the effect and the sampling design, e.g., fecal nitrogen failed to detect a small interaction between sex and day-of-spring, which pigments detected (Table 4). Fecal photosynthetic pigments may have more power to generate novel inferences into diet selection useful to herbivore ecologists because (*i*) photosynthetic pigments are indigestible (Reid et al. 1950; Smart et al. 1954; Troelsen 1961; Davis et al. 1968; Lane and Hassall 1996) and (*ii*) photosynthetic pigment concentrations are not apparently confounded by metabolic byproducts in feces (Deijs and Bosman 1955; Christianson and Creel 2009). These properties exempt fecal pigments from many of the strongest criticisms leveled at fecal nitrogen as an indicator of diet quality (Mould and Robbins 1981; Hobbs 1987; Robbins et al. 1987; Schwarm et al. 2009).

Fecal pigments have been successfully measured in a small number of free-ranging North American and African herbivores

Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.

Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.

(Christianson and Creel 2009). At this stage, we caution that any inference into diet quality from fecal pigments will be primarily dependent on the consistency of the positive relationship between chlorophyll content, digestibility, and nutrient content in forages (Table 1), but such positive relationships appear to commonly occur across a diversity of forages and landscapes (Van Soest 1982; Klein 1990; Wilmshurst et al. 1995; Chen et al. 1998; Larter and Nagy 2001). As with all fecal indices, interpretation of fecal pigments as an index of nutritional value is vulnerable to variation in intake rates, which can be considerable (Illius et al. 1999). Fecal pigments, at the very least, are a measure of effectiveness at which an individual herbivore concentrates chlorophyll or carotenoids in their feces through selection of photosynthetic plant tissue and efficient digestion of those tissues. Many questions in herbivore foraging ecology correspond to spatiotemporal scales where confounding variation in forage quality, digestive efficiency, and intake rates may be low or uncorrelated with biologically significant variation in photosynthetic tissue abundance or consumption (McNaughton 1985; Fryxell et al. 1988; Frank and McNaughton 1992; Augustine et al. 2003). We suggest that where useful inferences can be gained using qualitative or quantitative classification of greenness in herbivore forages or habitat (McNaughton 1985; Frank and McNaughton 1992; Augustine et al. 2003; Treydte et al. 2013), the application of fecal chlorophyll, a measure of the greenness of plants actually consumed by herbivores, should prove highly complementary and equally insightful. The growing application of satellite-based metrics to describe habitat quality, based largely on the optical qualities of chlorophyll at the landscape level (e.g., NDVI), might be advanced by understanding of how variation in plant phenology translates into diet shifts (Christianson and Creel 2009). Finally, climate change is altering plant phenology and spatiotemporal variability of primary production in a manner likely to be important to herbivore populations (Forchhammer and Post 2004; Berteaux et al. 2006). Fecal pigments may prove a powerful tool for detecting the effects of such forces on herbivores.

Acknowledgements

We thank the technicians who collected samples in the field: E. Borloz, S. Liley, C. Walstrom, and J. Winnie. This work was supported by grants from the National Science Foundation's Animal Behavior Program and GK-12 program.

References

Augustine, D.J., McNaughton, S.J., and Frank, D.A. 2003. Feedbacks between soil nutrients and large herbivores in a managed savanna ecosystem. Ecol. Appl. **13**: 1325–1337. doi:10.1890/02-5283.

Berteaux, D., Humphries, M.M., Krebs, C.J., Lima, M., McAdam, A.G., Pettorelli, N., Réale, D., Saitoh, T., Tkadlec, E., Weladji, R.B., and Stenseth, N.C. 2006. Constraints to projecting the effects of climate change on mammals. Clim. Res. **32**: 151–158. doi:10.3354/cr032151.

Blanchard, P., Festa-Bianchet, M., Gaillard, J.-M., and Jorgenson, J.T. 2003. A test of long-term fecal nitrogen monitoring to evaluate nutritional status in bighorn sheep. J. Wildl. Manage. **67**: 477–484. doi:10.2307/3802705.

Chen, H., Ma, J., Li, F., Sun, Z., Wang, H., Luo, L., and Li, F. 1998. Seasonal composition and quality of red deer *Cervus elaphus* diets in northeastern China. Acta Theriol. **43**: 77–94. doi:10.4098/AT.arch.98-6.

Christianson, D., and Creel, S. 2008. Risk effects in elk: sex-specific responses in grazing and browsing due to predation risk from wolves. Behav. Ecol. **19**: 1258–1266. doi:10.1093/beheco/arn079.

Christianson, D., and Creel, S. 2009. Fecal chlorophyll describes the link between primary production and consumption in a terrestrial herbivore. Ecol. Appl. **19**: 1323–1335. doi:10.1890/08-0987.1. PMID:19688938.

Christianson, D., and Creel, S. 2010. A nutritionally mediated risk effect of wolves on elk. Ecology, **91**: 1184–1191. doi:10.1890/09-0221.1. PMID:20462132.

Christianson, D., Klaver, R.W., Middleton, A., and Kauffman, M. 2013. Confounded winter and spring phenoclimatology on large herbivore ranges. Landsc. Ecol. **28**: 427–437. doi:10.1007/s10980-012-9840-2.

Creel, S., and Christianson, D. 2009. Wolf presence and increased willow consumption by Yellowstone elk: implications for trophic cascades. Ecology, **90**: 2454–2466. doi:10.1890/08-2017.1. PMID:19769124.

Creel, S., and Winnie, J.A. 2005. Responses of elk herd size to fine-scale spatial and temporal variation in the risk of predation by wolves. Anim. Behav. **69**: 1181–1189. doi:10.1016/j.anbehav.2004.07.022.

Creel, S., Winnie, J., Maxwell, B., Hamlin, K., and Creel, M. 2005. Elk alter habitat selection as an antipredator response to wolves. Ecology, **86**: 3387–3397. doi:10.1890/05-0032.

Davis, L.E., Jordan, R.M., and Marten, G.C. 1968. Evaluation of forage digestibility prediction equations using chromogen and nitrogen as fecal index indicators. Agron. J. **60**: 420–422. doi:10.2134/agronj1968.00021962006000040026x.

Deijs, W.B., and Bosman, M.S.M. 1955. Plant pigments in digestion trial studies. Recl. Trav. Chim. Pays-Bas Belg. **74**: 1207–1216. doi:10.1002/recl.19550741003.

Delgiudice, G.D., Seal, U.S., and Mech, L.D. 1991. Indicators of severe undernutrition in urine of free-ranging elk during winter. Wildl. Soc. Bull. **19**: 106–110.

Drent, R., Ebbinge, B., and Weijand, B. 1979. Balancing the energy budgets of arctic-breeding geese throughout the annual cycle: a progress report. Verh. Ornithol. Ges. Bayern, **23**: 239–264.

Forchhammer, M.C., and Post, E. 2004. Using large-scale climate indices in climate change ecology studies. Popul. Ecol. **46**: 1–12. doi:10.1007/s10144-004-0176-x.

Frank, D.A., and McNaughton, S.J. 1992. The ecology of plants, large mammalian herbivores, and drought in Yellowstone National Park. Ecology, **73**: 2043–2058. doi:10.2307/1941454.

Fraser, M.D., and Gordon, I.J. 1997. The diet of goats, red deer and South American camelids feeding on three contrasting Scottish upland vegetation communities. J. Appl. Ecol. **34**: 668–686. doi:10.2307/2404915.

Fryxell, J.M., Greever, J., and Sinclair, A.R.E. 1988. Why are migratory ungulates so abundant? Am. Nat. **131**: 781–798. doi:10.1086/284822.

Hobbs, N.T. 1987. Fecal indices to dietary quality: a critique. J. Wildl. Manage. **51**: 317–320. doi:10.2307/3801008.

Illius, A.W., Gordon, I.J., Elston, D.A., and Milne, J.D. 1999. Diet selection in goats: a test of intake-rate maximization. Ecology, **80**: 1008–1018. doi:10.1890/0012-9658(1999)080[1008:DSIGAT]2.0.CO;2.

Irwin, L.L., Cook, J.G., McWhirter, D.E., Smith, S.G., and Arnett, E.B. 1993. Assessing winter dietary quality in bighorn sheep via fecal nitrogen. J. Wildl. Manage. **57**: 413–421. doi:10.2307/3809442.

Klein, D.R. 1990. Variation in quality of caribou and reindeer forage plants associated with season, plant part, and phenology. Rangifer, **10**: 123–130. doi:10.7557/2.10.3.841.

Lane, S.J., and Hassall, M. 1996. Estimation of apparent metabolizability in herbivorous wildfowl with plant pigments. J. Wildl. Manage. **60**: 910–916. doi:10.2307/3802392.

Larter, N.C., and Nagy, J.A. 2001. Seasonal and annual variability in the quality of important forage plants on Banks Island, Canadian High Arctic. Appl. Veg. Sci. **4**: 115–128. doi:10.1111/j.1654-109X.2001.tb00242.x.

Leslie, D.M., and Starkey, E.E. 1985. Fecal indices to dietary quality of cervids in old-growth forests. J. Wildl. Manage. **49**: 142–146. doi:10.2307/3801860.

Leslie, D.M., Bowyer, R.T., and Jenks, J.A. 2008. Facts from feces: nitrogen still measures up as a nutritional index for mammalian herbivores. J. Wildl. Manage. **72**: 1420–1433. doi:10.2193/2007-404.

Lichtenthaler, H. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. **148**: 350–382. doi:10.1016/0076-6879(87)48036-1.

Lin, L., Dickhoefer, U., Müller, K., Wurina, and Susenbeth, A. 2011. Grazing behavior of sheep at different stocking rates in the Inner Mongolian steppe, China. Appl. Anim. Behav. Sci. **129**: 36–42. doi:10.1016/j.applanim.2010.11.002.

Lowry, J.B., and Schlink, A.C. 1995. Chlorophyll-derived faecal pigment as an indicator of feed selection in dry-season tropical pastures. Trop. Agric. **72**: 303–307.

Massey, B.N., Weckerly, F.W., Vaughn, C.E., and McCullough, D.R. 1994. Correlations between fecal nitrogen and diet composition in free-ranging black-tailed deer. Southwest. Nat. **39**: 165–170. doi:10.2307/3672242.

McNaughton, S.J. 1976. Serengeti migratory wildebeest: facilitation of energy flow by grazing. Science, **191**: 92–94. doi:10.1126/science.191.4222.92. PMID:17834943.

McNaughton, S.J. 1979. Grazing as an optimization process: grass ungulate relationships in the Serengeti. Am. Nat. **113**: 691–703. doi:10.1086/283426.

McNaughton, S.J. 1985. Ecology of a grazing ecosystem: the Serengeti. Ecol. Monogr. **55**: 259–294. doi:10.2307/1942578.

Misra, M.K., and Misra, B.N. 1981. Seasonal changes in leaf area index and chlorophyll in an Indian grassland. J. Ecol. **69**: 797–805. doi:10.2307/2259637.

Mould, E.D., and Robbins, C.T. 1981. Nitrogen metabolism in elk. J. Wildl. Manage. **45**: 323–334. doi:10.2307/3807915.

Murray, M.G., and Brown, D. 1993. Niche separation of grazing ungulates in the Serengeti: an experimental test. J. Anim. Ecol. **62**: 380–389. doi:10.2307/5369.

Murray, M.G., and Illius, A.W. 2000. Vegetation modification and resource competition in grazing ungulates. Oikos, **89**: 501–508. doi:10.1034/j.1600-0706.2000.890309.x.

Pettorelli, N., Vik, J.O., Mysterud, A., Gaillard, J.-M., Tucker, C.J., and Stenseth, N.C. 2005. Using the satellite-derived NDVI to assess ecological responses to environmental change. Trends Ecol. Evol. **20**: 503–510. doi:10.1016/j.tree.2005.05.011. PMID:16701427.

Rabinowitch, E., and Govindjee. 1969. Photosynthesis. Wiley, New York.

R Development Core Team. 2012. R: a language and environment for statistical computing. Version 2.15.12 [computer program]. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.r-project.org/.

Reid, J.T., Woolfolk, P.G., Richards, C.R., Kaufmann, R.W., Loosli, J.K., Turk, K.L., Miller, J.I., and Blaser, R.E. 1950. A new indicator method for the determination of digestibility and consumption of forages by ruminants. J. Dairy Sci. **33**: 60–71. doi:10.3168/jds.S0022-0302(50)91865-0.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C., and Mautz, W.W. 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology, **68**: 98–107. doi:10.2307/1938809.

Ryan, S.J., Knechtel, C.U., and Getz, W.M. 2007. Ecological cues, gestation length, and birth timing in African buffalo (*Syncerus caffer*). Behav. Ecol. **18**: 635–644. doi:10.1093/beheco/arm028.

Ryan, S.J., Cross, P.C., Winnie, J., Hay, C., Bowers, J., and Getz, W.M. 2012. The utility of normalized difference vegetation index for predicting African buffalo forage quality. J. Wildl. Manage. **76**: 1499–1508. doi:10.1002/jwmg.407.

Schwarm, A., Schweigert, M., Ortmann, S., Hummel, J., Janssens, G.P.J., Streich, W.J., and Clauss, M. 2009. No easy solution for the fractionation of faecal nitrogen in captive wild herbivores: results of a pilot study. J. Anim. Physiol. Anim. Nutr. **93**: 596–605. doi:10.1111/j.1439-0396.2008.00842.x.

Shrader, A., Owen-Smith, N., and Ogutu, J. 2006. How a mega-grazer copes with the dry season: food and nutrient intake rates by white rhinoceros in the wild. Funct. Ecol. **20**: 376–384. doi:10.1111/j.1365-2435.2006.01107.x.

Sinclair, A.R.E., Mduma, S.A.R., and Arcese, P. 2000. What determines phenology and synchrony of ungulate breeding in Serengeti? Ecology, **81**: 2100–2111. doi:10.1890/0012-9658(2000)081[2100:WDPASO]2.0.CO;2.

Smart, W.W.G., Matrone, G., and Smart, V.W. 1954. Feed digestibility: use of copper derivatives of chlorophylls in ratio method for estimating digestibility of forages. J. Agric. Food Chem. **49**: 1331–1332. doi:10.1021/jf60046a006.

Sparks, D.R., and Malechek, J.C. 1968. Estimating percentage dry weight in diets using a microscopic technique. J. Range Manage. **21**: 264–265. doi:10.2307/3895829.

Szymczak-Żyla, M., Wawrzyniak-Wydrowska, B., and Kowalewska, G. 2006. Products of chlorophyll *a* transformation by selected benthic organisms in the Odra Estuary (southern Baltic Sea). Hydrobiologia, **554**: 155–164. doi:10.1007/s10750-005-1016-5.

Treydte, A.C., Baumgartner, S., Heitkönig, I.M.A., Grant, C.C., and Getz, W.M. 2013. Herbaceous forage and selection patterns by ungulates across varying herbivore assemblages in a South African savanna. PloS ONE, **8**: e82831. doi:10.1371/journal.pone.0082831. PMID:24358228.

Troelsen, J.E. 1961. Plant chromogens as an indicator in estimating the digestibility of forages crops by sheep. Can. J. Plant Sci. **41**(4): 732–739. doi:10.4141/cjps61-109.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. Cornell University Press, Ithaca, N.Y.

Wehausen, J.D. 1995. Fecal measures of diet quality in wild and domestic ruminants. J. Wildl. Manage. **59**: 816–823. doi:10.2307/3801962.

Wilmshurst, J.F., Fryxell, J.M., and Hudson, R.J. 1995. Forage quality and patch choice by wapiti (*Cervus elaphus*). Behav. Ecol. **6**: 209–217. doi:10.1093/beheco/6.2.209.

Winnie, J., and Creel, S. 2007. Sex-specific behavioural responses of elk to spatial and temporal variation in the threat of wolf predation. Anim. Behav. **73**: 215–225. doi:10.1016/j.anbehav.2006.07.007.

Wofford, H., Holechek, J.L., Galyean, M.L., Wallace, J.D., and Cardenas, M. 1985. Evaluation of fecal indices to predict cattle diet quality. J. Range Manage. **38**: 450–454. doi:10.2307/3899720.

Can. J. Zool. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 01/14/15
For personal use only.