

Fecal chlorophyll describes the link between primary production and consumption in a terrestrial herbivore

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Abstract. Spatiotemporal variation in primary productivity is known to have strong and far-reaching effects on herbivore ecology, but this relationship is often studied indirectly at broad scales, in part due to the difficulty in measuring selection for green biomass by individual animals. 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 in feces from elk (*Cervus elaphus*) experiencing large fluctuations in primary production in the winter to spring transition over three years. We compared temporal trends in fecal chlorophyll with trends in fecal nitrogen, grass chlorophyll, grass digestible nitrogen, and landscape-level primary productivity (as described by the normalized difference vegetation index or NDVI). We also directly examined the relationship between fecal chlorophyll and NDVI. Temporal trends in fecal chlorophyll were strong and well described by piecewise regression (adjusted coefficient of determination, $r_a^2 = 0.881\text{--}0.888$), 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 the chlorophyll and digestible nitrogen concentration of forage grasses collected directly from elk feeding sites. Fecal chlorophyll also tracked broad temporal patterns in fecal nitrogen and NDVI, but discrepancies between the indexes may highlight preferences or constraints on selectivity for green biomass in elk. Spatially and temporally matched NDVI and fecal chlorophyll estimates were uncorrelated until NDVI reached approximately half its seasonal range. Combined, these data describe important patterns in selection for nutritious, green biomass in a temperate herbivore that would be difficult to study without data on fecal chlorophyll. Fecal chlorophyll produced novel and precise descriptions of (and detected large interannual differences in) winter length, severity, and the 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 aspect of foraging ecology in terrestrial herbivores and may be particularly powerful for studying climate effects in seasonal environments.

Key words: *Cervus elaphus*; chlorophyll in herbivore feces; climate and phenological effects on ruminants; foraging during spring green-up; NDVI; nitrogen; primary production; season; selection for photosynthetic biomass.

INTRODUCTION

Primary production, the photosynthetic conversion of carbon dioxide to biomass, creates the energetic foundation of nearly every community on earth. Spatial and temporal variation in primary production has dominating effects on the ecology, evolution, and population dynamics of herbivores (Olf et al. 2002, Fritz and Loison 2006). The vital rates of herbivores are tightly coupled to the quality and quantity of forage plants, and their life histories are structured around seasonal patterns of plant growth (McNaughton 1985, McNaughton and Georgiadis 1986, Fryxell et al. 1988,

Sinclair et al. 2000, Ryan et al. 2007). 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).

For herbivores in seasonal environments, green, photosynthetic vegetation is more nutritious than dormant vegetation, and is preferentially selected (Wilmshurst et al. 1999, Murray and Illius 2000, Macandza et al. 2004, Shrader et al. 2006). The greenness of tissues, entire plants, or patches of vegetation is directly and causally related to the production and availability of carbohydrates, nitrogen, and other nutrients that are often limiting for herbivores, as green pigmentation indicates the presence of chlorophyll, the primary pigment involved in photosyn-

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thesis (Misra and Misra 1981, Frank and McNaughton 1993, McNaughton et al. 1997, Augustine et al. 2003, Mehaffey et al. 2005). Given these basic relationships, it is not surprising that a large body of heavily cited research confirms that significant variation in herbivore nutrition and behavior can be explained by simple descriptions of the spatial and temporal variation in the greenness (or chlorophyll concentration) of vegetation (McNaughton 1976, Frank and McNaughton 1992, Frank et al. 1998, Sinclair et al. 2000).

There are several techniques to quantify the greenness or the chlorophyll concentration of vegetation (Hardwick and Baker 1973, McNaughton 1976, Misra and Misra 1981). Satellite-based measures (e.g., NDVI), in particular, are highly available and frequently used by ecologists in current research (Pettorelli et al. 2005). These techniques describe the vegetation available to herbivores and have proven quite powerful to describe the broad effects of variation in primary production on herbivores (Loe et al. 2005, Pettorelli et al. 2006, 2007, Mysterud et al. 2007). For more specific questions about foraging behavior, however, these methods would require the assumption that in situ measurements of the vegetation correlate directly with the vegetation consumed by herbivores. This assumption would be destabilized by the selectivity of the taxa being studied and the spatiotemporal resolution of the methods used to describe primary productivity. For example, with NDVI, the data are largely limited to a spatial resolution of $\geq 900 \text{ m}^2$ and a temporal resolution $\geq 10 \text{ d}$. This scale relates to few foraging decisions in small herbivores (Hughes et al. 1994, Hodges and Sinclair 2005), while even foraging by the largest herbivores varies significantly within these scales (Augustine and McNaughton 1998, Fortin et al. 2005). These in situ techniques are quite powerful for answering broad questions about the relationship between producers and consumers. The fine-scale selection by individual herbivores for green biomass is a closely related question that explores this fundamental relationship further, but requires a different approach.

For describing diet selection by individuals at finer scales, fecal indices are often employed (Holechek et al. 1982, Wehausen 1995, Blanchard et al. 2003). Yet despite broad recognition of its fundamental importance at broader scales, there is currently no fecal index that directly describes the greenness of the diet. Aquatic ecologists have found that the concentration of chlorophyll in the feces of aquatic herbivores can be used to describe the consumption of primary production and explore trophic interactions at fine scales (Bathmann and Liebezeit 1986, Szymczak-Zyla et al. 2006). Surprisingly, this approach has never been used in terrestrial ecology, despite experimental evidence from captive birds and domestic ruminants that shows that photosynthetic pigments are excreted in the feces in proportion to their rate of consumption (Reid et al. 1950, Deijs and Bosman 1955, Lowry and Schlink 1995, Lane and

Hassall 1996). Further, measurements of fecal chlorophyll can be made quickly and inexpensively for large numbers of samples with a high degree of precision. We explored whether the light absorption by chlorophyll could be measured in the feces of a wild, terrestrial herbivore, and we explored how this index might be used to describe relationships between herbivory and primary production in a temperate ecosystem.

METHODS

Study area and data collection

The Upper Gallatin elk herd (1100 ± 260 animals [mean \pm SD], from aerial counts, Montana Fish Wildlife and Parks, 2003–2005) is a migratory population that occupies several areas of wilderness in southwestern Montana, moving from high-altitude ($>2400 \text{ m}$ above sea level) summer ranges in June to lower (1975–2200 m) winter ranges in November, traveling as far as 50 km (Brazda 1953). Foraging behavior, mass dynamics, migration, and parturition appear to be closely linked with spatiotemporal variation in environmental conditions in this population (Johnson 1951, Brazda 1953, Greer and Howe 1964, Peek and Lovaas 1968, Creel et al. 2005, Christianson and Creel 2008). Thus, winter and spring conditions are critical for determining the synchrony between elk life history and plant phenology, and are also strong drivers of elk population dynamics (Houston 1982, Singer et al. 1997, Taper and Gogan 2002). We collected the following data on elk foraging, nutrition, and environmental conditions from December through May, in 2004, 2005, and 2006. For further descriptions of this study population and our sampling design, see Christianson and Creel (2008).

Fecal and forage samples.—We located elk groups every 14 d in each of three sites occupied by the Upper Gallatin elk herd, and collected 10 fecal samples ($\sim 30 \text{ mL}$) from 10 fresh ($<24 \text{ h}$ old) 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 three sites 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, although forested locations were primarily small and isolated and/or low-density conifer stands (Appendix A, and see Patten 1963, Creel et al. 2005, Shoutis 2007 for detailed descriptions of the vegetation). 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 (Cook 2002, Christianson and Creel 2007), grazing in open habitats is the primary foraging strategy used in the Upper Gallatin

(Constan 1967, Creel et al. 2005, Christianson and Creel 2008). Microhistological diet analysis of elk fecal samples confirmed that grass dominated the diet throughout the course of this study; $72.8\% \pm 19.0\%$ (mean \pm SD) of plant fragments in 984 fecal samples were grass. To assess the nutritional quality and phenology of this important forage, we collected vegetation samples (20.12 ± 12.56 g) 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 Dalebout 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 ($93.8\% \pm 13.7\%$), and we refer to forage samples as grass, for simplicity. Grass samples were stored at -20°C , dried for 48 h 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 and the initial reason for this processing step (Creel et al. 2007). A 2.0-g subsample of feces or grass was dried in a rotary evaporator for 12 h. A 0.2-g portion of dried matter was weighed to the nearest 0.001 g and boiled in 95% ethanol for 15 min. 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, Washington, USA. For grasses, we combined these measurements into a single nutritional index, digestible nitrogen (nitrogen \times in vitro dry matter digestibility), to reflect forage quality, specifically, the availability of nitrogen to a ruminant (Mould and Robbins 1981).

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 aboveground 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, albeit at different scales and in different contexts. We acquired NDVI values for the study area from the Global Land Cover Facility at the University of Maryland, College

Park, USA (information *available online*).² 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 -0.25 to 1.0 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 subsampling and averaging 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 (Appendix A). 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 (Appendix A). We used the locations of the 2005 fecal collections (when conditions were similar, see *Results*) to select values from the 2004 NDVI data set for averaging. Raw NDVI data are prone to errors (Pettorelli et al. 2005), and we excluded NDVI values that were extreme outliers (>2.5 standard deviations) 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 and extracting fecal samples over the winter–spring transition, we perceived a qualitative difference in the seasonal coloration of feces. 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 diet selection and fine-scale behavioral responses. Therefore, we reviewed procedures for the quantification of photosynthetic pigments in biological preparations (Arnon 1949, Parsons and Strickland 1963, Goodwin 1965, 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 selection for green biomass. First, we compared the absorption spectra (the intensity of light absorption across all the wavelengths of the visible light

² (<http://www.landcover.org>)

spectrum) of elk feces and forage grasses to the spectra of pure photosynthetic pigments (Sigma Aldrich Company, 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 do so. Elk feces should show this same seasonal pattern if fecal pigment concentration can be used to reliably describe consumption of green biomass.

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 380 nm to 780 nm for a 200- μ L 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 twofold dilution series of a pooled sample from 20 fecal extracts, from undiluted to 1024 \times 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. 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 also showed the expected patterns if photosynthetic pigments were present in the diet.

Primary measurement of chlorophyll in fecal and forage extracts.—Upon validation of our laboratory methods discussed above (see *Results*), we subsequently measured light absorption for all samples at a single wavelength, 666 nm (OD_{666}) and used this measurement to explore green biomass consumption by elk in this system. We chose this wavelength because chlorophyll *a* absorbs light most strongly at 666 nm in methanol, whereas most other pigments do not (Goodwin 1965, Lichtenthaler and Wellburn 1983). Additionally, a peak of absorption

in herbivore fecal extracts near 666 nm has been found by others (Smart 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. Intra-assay 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 $OD = 0.2 \times (OD \times [g \text{ dry matter}]^{-1})$ (Greenhalgh and Corbett 1960), where the mass 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 provided estimates of the relative concentrations of chlorophyll that were directly comparable to one another and would allow for direct comparisons with other systems. To compare results from our study with those that estimated absolute quantities of chlorophyll, all ODs 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 666 nm in plants and feces as chlorophyll or greenness (Grant 1971). We used these OD_{666} values to explore the relationship between fecal chlorophyll and plant phenology in several different ways.

Data analysis

Describing temporal patterns.—We compared three types of regression model for describing seasonal trends in each of our dependent variables (fecal chlorophyll, fecal nitrogen, grass chlorophyll, grass nitrogen, and NDVI). As discussed above, each of these variables should be strongly affected by plant phenology as winter progresses into the growing season, but the shape of this relationship might vary across indices and ecological circumstances (Frank and McNaughton 1992, Massey et al. 1994, Mysterud et al. 2001, Pettorelli et al. 2007). 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 regressions. We considered three functional relationships between each of our dependent variables and our independent variable (Julian day 1 = 1 January): linear, linear with a quadratic term (hereafter, quadratic), or a discontinuous, piecewise regression

(Toms and Lesperance 2003). Our piecewise regression model takes the following form:

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

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 ($x \geq \text{breakpoint}$) 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 examination of the relationship between primary production and green biomass consumption, we also regressed fecal chlorophyll directly onto NDVI at the time of each fecal collection. We maintained our original stratification by comparing fecal chlorophyll to the NDVI values within a site at the time of fecal collection. 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 would describe a threshold NDVI value where the linear relationship between fecal chlorophyll and NDVI might change.

Statistical analysis.—We fit all models by least squares estimation using the GLM module of Statistica (StatSoft Incorporated, Tulsa, Oklahoma, USA). 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_a^2). 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. 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. 1; Appendix B). Black and grizzly bears, which are omnivores, showed peaks of light absorption at the same wavelengths as herbivores, but at lower magnitudes, as expected (Fig. 1D). Extracts from forage grasses produced higher spectrophotometric peaks in spring than in winter (Fig. 1B, $t = 11.19$, $P < 0.0001$) as did extracts from elk feces (Fig. 1C, $t = 13.39$, $P < 0.001$). 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. 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

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. 2), and piecewise regression fit this pattern well ($r_a^2 = 0.887, 0.881, 0.888$, 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 ($OD_{666}, 0.026 \pm 0.0006$ [mean \pm SE], $n = 786$), 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 rise in fecal chlorophyll as estimated by the breakpoint in the piecewise regression (Fig. 2; Appendix C) was highly precise in all winters. The onset of this rapid rise ($\pm 95\%$ CI) occurred on 28 March (± 2.3 d) in 2004, 1 April (± 2.0 d) in 2005, and 21 April (± 1.3 d) in 2006. Fecal chlorophyll increased rapidly after these dates, but at significantly different rates each year (Table 1; Appendix C). Temporal changes in fecal chlorophyll coincided

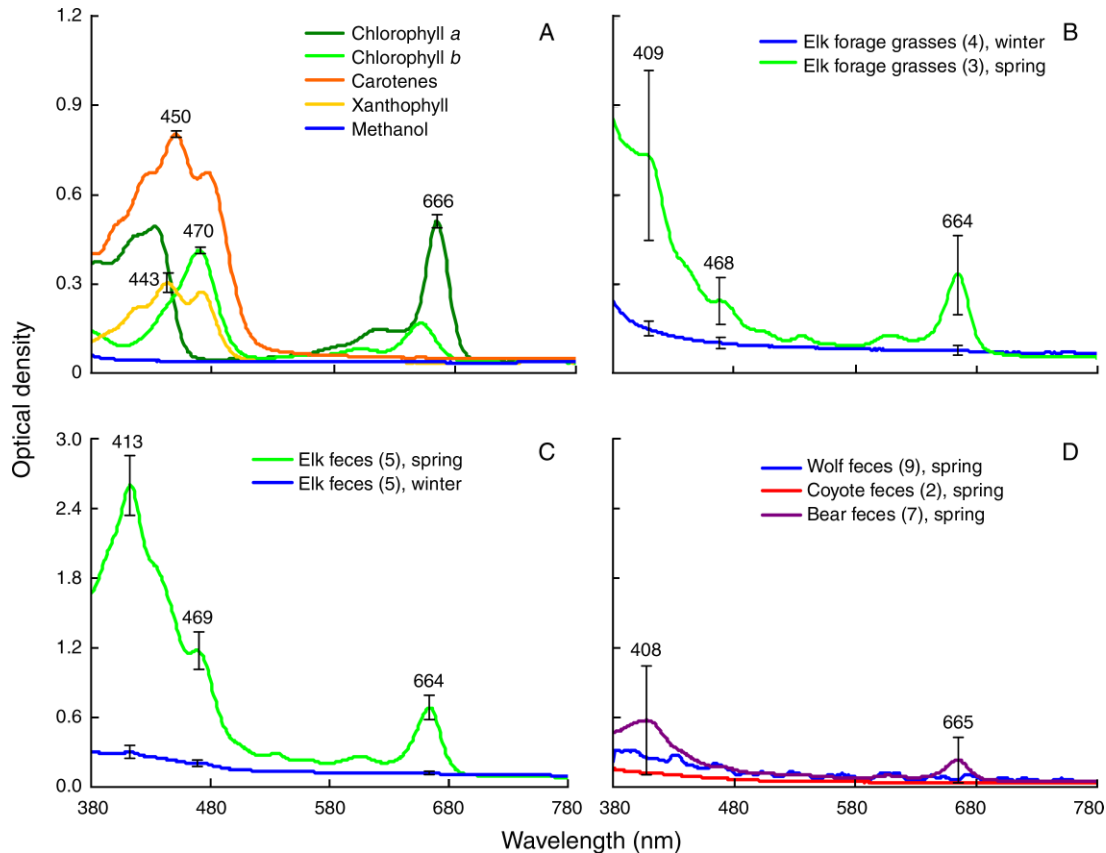


FIG. 1. Light absorption from 380 to 780 nm and 95% CI (resolution, 1 nm) at local maxima for (A) pure preparations of photosynthetic pigments and 100% methanol (used as an extraction solvent), (B) extracts from forage grasses in winter and spring, (C) fecal extracts from elk in winter and spring, and (D) fecal extracts from carnivores sympatric with elk in spring. Light absorption is averaged for each species (sample size in parentheses). All pigment samples were run in triplicate. Similarities in light absorption patterns between pigments, plants, elk feces, and sympatric omnivores (D, bears) confirmed that photosynthetic pigments from plants survive passage through the digestive tract and can be measured without interference.

closely with the spring melt-out (Fig. 2), as documented by snow–water equivalents at three snowpack telemetry (SNOTEL) towers in the Upper Gallatin (U.S. Natural Resources Conservation Service, elevation 2630 ± 140 m above sea level [mean \pm SD]) and average weekly temperatures from a weather station located within the study area (U.S. National Oceanic and Atmospheric Administration, 2010 m).

Although our data collection did not consistently extend into late spring when fecal chlorophyll concentrations probably reach an asymptote, the chlorophyll concentration of the final three fecal collections in the spring of 2004 (19–24 May, $n = 37$) was slightly less than the three preceding collections (6–14 May, $n = 48$), indicating that diet greenness reached an asymptote near these dates (OD_{666} , 0.683 ± 0.021 vs. 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). A post hoc piecewise regression estimated a secondary breakpoint (\pm SE) 44 days after fecal chlorophyll first began to rise, or 12 May 2004 (± 2.4 d, $t_{175} = 56.31$, $P <$

0.001) indicating the date when fecal chlorophyll may have stabilized at an annual maxima. This restricted analysis in late spring is highly preliminary and is intended only to demonstrate a logical extension of this approach.

Comparing fecal chlorophyll with temporal trends in fecal nitrogen

Due to their cost (\$US17 per sample), our sample sizes for fecal nitrogen were smaller than for fecal chlorophyll in 2004 ($n = 238$, 62% of total) and 2005 ($n = 310$, 78%). Rerunning piecewise regressions 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. 3A), and AIC values for piecewise regressions remained more informative 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 ($r_a^2 = 0.387$ – 0.856), but described a similar pattern in elk diet quality, with low values in midwinter and high values in spring. In 2005

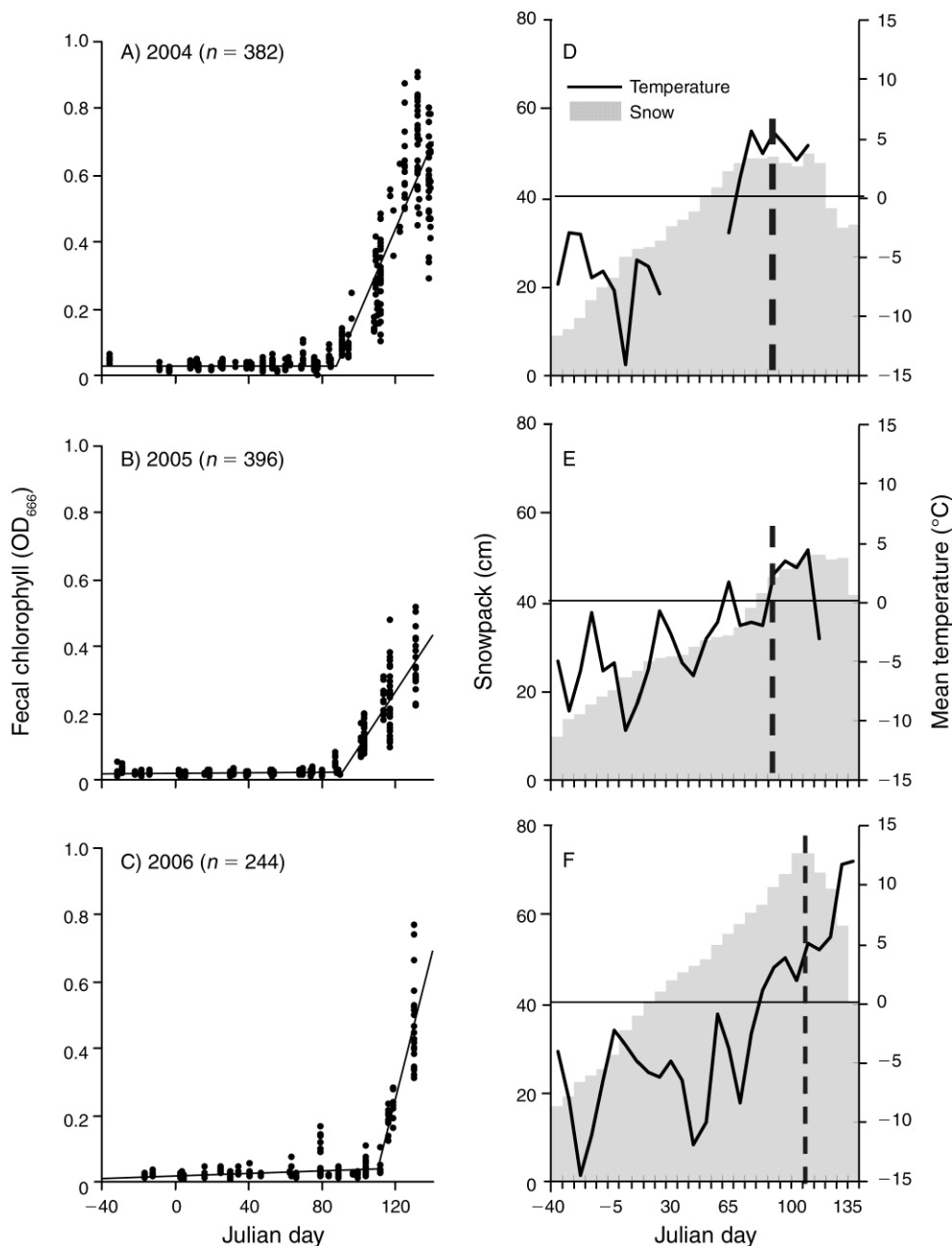


FIG. 2. Temporal pattern of chlorophyll concentration in elk feces (optical density of fecal extracts at 666 nm) over three winters and correlation with local weather (Julian day 1 = 1 January). Note that sample sizes at each fecal collection were approximately equal, and fecal chlorophyll values are strongly overlapping in winter in this figure. Piecewise regression (solid lines, A–C) fit the data well as fecal chlorophyll (data points) remained stable and low in winter but began to rise rapidly after the cessation of snow accumulation (gray bars, D–F) at high elevation (indicating reduced snow cover at elk foraging sites at lower elevation) and daily temperatures (solid line, D–F) rose above 0°C. The position of dashed vertical lines in D–F (line width denotes 95% CI) coincides with the estimated breakpoint in the piecewise regression for each winter (28 March 2004, 1 April 2005, and 21 April 2006). Gaps in weekly average temperature are original to the data set.

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 in fecal nitrogen (Appendix D). In 2004, sampling extended later into the growing season, which produced high fecal

nitrogen estimates with strong leverage. (Fourteen of the highest concentrations came from the final two collections in May 2004.) Consequently, piecewise regression was selected by AIC in 2004. (Linear and quadratic regressions were >54 units worse.) When we dichotomized the data from each year into winter and spring

TABLE 1. Standardized coefficient estimates of the effects of Julian day on elk feces, grass forage, and normalized difference vegetation index (NDVI) after the estimated breakpoint in piecewise regressions for three years.

Year (winter)	Nonlinear components†	Fecal chlorophyll	Fecal nitrogen	Grass chlorophyll	Grass digestible nitrogen	NDVI
2004	$b_1 + b_2$ breakpoint	0.941‡ 87.7	0.868 75.3			0.809§ 66.3
2005	$b_1 + b_2$ breakpoint	0.948 90.8	0.797§ 47.7	0.858 87.0	0.692 84.4	0.720§ 23.5
2006	$b_1 + b_2$ breakpoint	0.971 110.4	0.578§ 57.0			0.835 81.6

† For all values of the independent variable greater than the estimated breakpoint in a piecewise regression, the relationship between the dependent variable and independent variable is equal to $b_1 + b_2$. The breakpoint estimates the Julian day (Julian day 1 = 1 January) when the relationship between the Julian day and the independent variable has changed.

‡ When samples collected after 12 May (when chlorophyll concentration stabilized) were not considered, $b_1 + b_2 = 1.033$.

§ Quadratic regressions onto Julian day were selected by AIC or were within two AIC units of piecewise regressions.

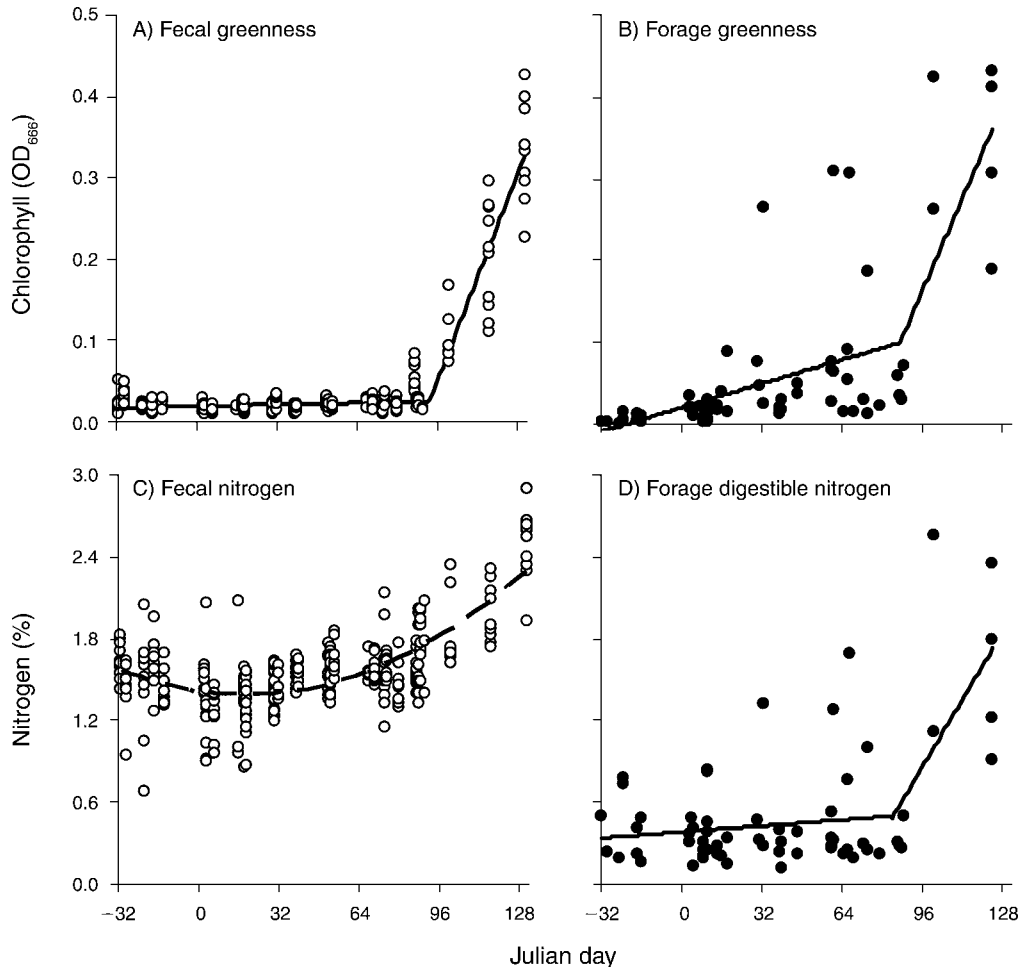


FIG. 3. The relationship between nitrogen and chlorophyll content and day of winter for elk fecal samples ($n = 310$) and elk forage grasses ($n = 64$) in 2005. Note that sample sizes at each fecal collection were approximately equal and that fecal chlorophyll values are strongly overlapping in winter in this figure. Piecewise regressions (solid lines) explained more variation than quadratic or simple linear relationships with day of winter except in graph C (dashed line), fecal nitrogen, where a quadratic regression was selected by AIC.

samples using the breakpoint in fecal chlorophyll, the linear correlation between fecal nitrogen and fecal chlorophyll was positive but weak in winter (5.43 ± 0.51 [coefficient \pm SE], $r_a^2 = 0.144$, $F_{1,678} = 115.26$, $P < 0.001$), and positive but strong in spring (1.95 ± 0.14 , $r_a^2 = 0.626$, $F_{1,111} = 188.56$, $P < 0.001$).

Comparing fecal chlorophyll with temporal trends in grass nitrogen and chlorophyll

At elk foraging sites, grass nitrogen ($1.12 \pm 0.07\%$ [mean \pm SE], $n = 109$), digestibility, ($38.37 \pm 1.24\%$), and chlorophyll content (0.047 ± 0.009 OD₆₆₆) were low throughout the study period, but highest in spring. Although forage sampling in 2004 ($n = 17$) and 2006 ($n = 22$) was limited and did not extend past Julian day 95 and 96, respectively, pooling these winter collections across years showed no change in digestible nitrogen ($r_a^2 = -0.012$, $F_{1,38} = 0.534$, $P = 0.469$), and a weak increase in chlorophyll content throughout the winter ($r_a^2 = 0.008$, $F_{1,37} = 4.289$, $P = 0.045$). This weak increase in chlorophyll was driven by a single grass sample in late winter with high leverage (day 92), and removing this point showed no detectable change in grass chlorophyll content over winter ($r_a^2 = -0.001$, $F_{1,36} = 0.974$, $P = 0.330$), similar to fecal chlorophyll. We restricted model selection to 2005 when forage sample sizes were larger ($n = 64$) and sampling extended longer into the growing season. Temporal patterns of chlorophyll and nitrogen in forage grasses in 2005 were best described by piecewise regressions ($r_a^2 = 0.576$ and 0.401 , respectively, Appendix E). 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 (Table 1, Fig. 3). Digestible nitrogen and chlorophyll content in grasses were more strongly correlated than they were in feces (4.21 ± 0.21 [coefficient \pm SE], $r_a^2 = 0.807$, $F_{1,100} = 424.58$, $P < 0.001$). Chlorophyll content in forage grasses rose more quickly than nitrogen, as revealed by standardized regression coefficients (Table 1).

Comparing fecal chlorophyll with temporal trends in primary productivity

Annual range for NDVI was from -0.09 to 0.90 , while the range over just the winter–spring period spanning fecal collections varied from -0.04 to 0.66 (71% of the annual range). Temporal trends in NDVI were similar to patterns in fecal chlorophyll concentration (Appendix F). Piecewise regressions of NDVI on Julian day usually performed better than quadratic and linear models (Appendix G); however, in 2004 and 2005, quadratic models were within 2 AIC units of the nonlinear, piecewise regression (Appendix G). Both piecewise and quadratic relationships fit well across all winters ($r_a^2 = 0.535$ – 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

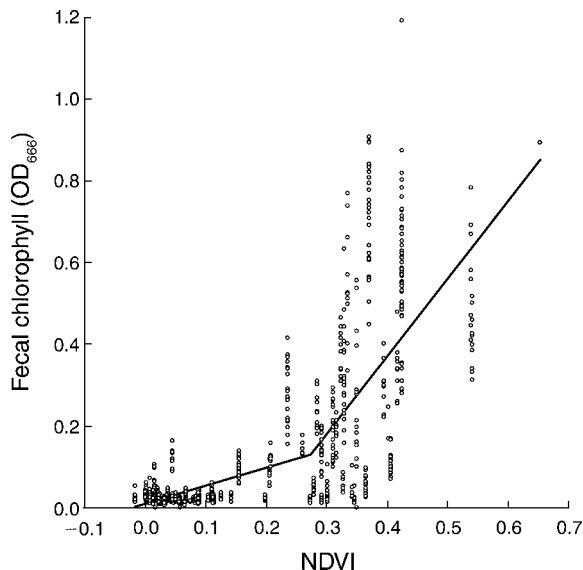


FIG. 4. The relationship between the chlorophyll content of elk feces collected December–May, 2004–2006, and average NDVI (normalized difference vegetation index) across the site at the time of each fecal collection. Note that sample sizes at each fecal collection were approximately equal, and low fecal chlorophyll values are strongly overlapping in this figure. The greater information content of the piecewise relationship over quadratic or linear relationships, as determined by AIC, suggests a nonlinear response between the abundance of green biomass and its consumption by elk.

detectably differ from 0, $P > 0.2$), mirroring the pattern seen in fecal chlorophyll (Appendices B and E). The estimated onset of green-up from NDVI values was 21.4, 67.3, and 28.8 d 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, but the rate of green-up was slowest in 2005 and fastest in 2006, similar to the pattern in elk fecal chlorophyll (Table 1). While the growing season arrived earlier in 2005 than 2004 (23.5 ± 6.5 and 66.3 ± 2.6 d [Julian day \pm SE], 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 1; Appendices B and E).

Relationship between primary productivity and fecal chlorophyll

We found a strong, direct relationship between NDVI and fecal chlorophyll (Fig. 4; Appendix H). Critically, fecal chlorophyll did not respond to increasing NDVI in a linear or quadratic fashion, and the piecewise regression was 127 and 14 AIC units better, respectively (Appendix H). Fecal chlorophyll increased weakly across a broad range of low NDVI values, then increased at a much faster rate once NDVI values reached 0.27, approximately half the seasonal range in NDVI (Fig. 4, Appendix H). The piecewise regression

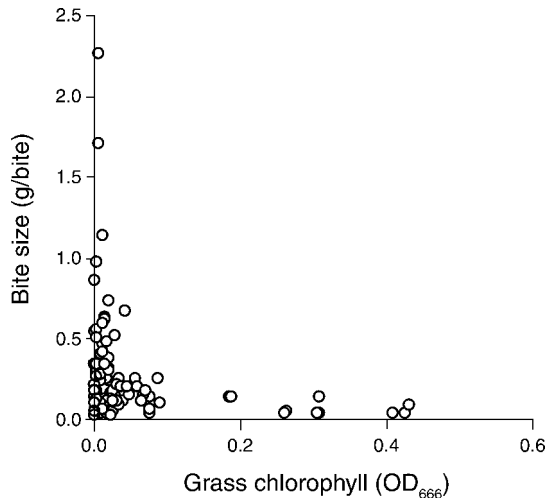


FIG. 5. Bite size (grams of dry matter) and chlorophyll content for grasses ($n = 100$) collected from elk foraging sites in winter and spring 2004–2006.

on NDVI explained slightly more than half the variation in fecal chlorophyll ($r_a^2 = 0.568$). Fecal collections from partially 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.

DISCUSSION

We concluded that the optical density of fecal extracts at 666 nm could be measured reliably and correlated well with the availability of green biomass to a terrestrial herbivore in a temperate ecosystem. This largely validates an approach that, until now, has only been applied to aquatic herbivores (Bathmann and Liebezeit 1986). Our results build on experimental data from domestic ruminants, which conclude that chlorophyll and its metabolites survive passage through the digestive tract at high rates of survival and that light absorption at 666 nm indexes their concentration with few confounding factors (Smart et al. 1953, Deijs and Bosman 1955, Boudon et al. 2002). This simple, noninvasive method can be conducted at low cost and great speed (per sample, ~\$US 0.85 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). Previous work has clearly established the strong link between plant phenology and herbivores in seasonal environments (McNaughton 1976, Wilmshurst et al. 1999, Sinclair et al. 2000, Pettorelli et al. 2007). Our results present a useful tool to explore more specific questions in a manner that allows one to examine the direct consumption of green, photosynthetic biomass by individual animals.

Several of our results suggested that the presence of green biomass did not always coincide with increased

fecal chlorophyll (Fig. 4; Appendix F). Indeed, while harvesting forage samples at elk feeding sites, green grass could always be found (by ourselves and by elk) prior to the dramatic annual rise in fecal chlorophyll, but it did not appear to significantly affect fecal chlorophyll. 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. 3B), but our efforts to mimic elk foraging using grab samples precluded us from selectively harvesting only the green tissue without dormant culms and leaves from the previous growing season. Additionally, our attempts at harvesting new growth at elk foraging sites in early spring was inefficient at first emergence when shoots were short. Subsequently, the greenest “bites” we could manually harvest were often the smallest (Fig. 5). Fecal chlorophyll suggested 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 (Appendix F). These data suggest, in several different ways, that large herbivores like elk may not be capable of exploiting sparse green biomass efficiently until primary productivity reaches a threshold, an inference that was revealed in our data by the measurement of fecal chlorophyll. While such a finding is not necessarily new or surprising, this approach demonstrates what can be learned about herbivore selectivity and foraging constraints using a straightforward comparison of the availability of green vegetation (through NDVI or forage sampling) and the consumption of green biomass as measured by fecal chlorophyll.

Finally, if winter conditions and plant phenology influence diet, survival, and reproduction in temperate herbivores (Albon and Langvatn 1992, Adams 2003, Stenseth et al. 2003, Mysterud and Ostbye 2006), then quantifying consumption of green biomass through fecal chlorophyll 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, 2007, Mysterud et al. 2007). Here, we uniquely described these same parameters, but *as they were experienced by a large, grazing herbivore* using simple, piecewise regressions of fecal chlorophyll on time. This approach revealed biologically significant interannual differences in nearly every parameter (e.g., the onset of fecal green-up varied by as much as 22 d; Appendix C). Fecal chlorophyll may be quite powerful for exploring how

environmental change impacts foraging behavior, life histories, and trophic interactions in terrestrial communities.

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APPENDIX A

Study area in the Upper Gallatin Canyon of southwestern Montana and the northwest corner of Yellowstone National Park (*Ecological Archives* A019-053-A1).

APPENDIX B

Light absorption from 380 to 780 nm (resolution, 1 nm) for fecal extracts from North American and African mammals (*Ecological Archives* A019-053-A2).

APPENDIX C

Comparison of linear, quadratic, and piecewise regressions of elk fecal chlorophyll on Julian day over three winter–spring transitions (*Ecological Archives* A019-053-A3).

APPENDIX D

Comparison of linear, quadratic, and piecewise regressions of elk fecal nitrogen on Julian day over three winter–spring transitions (*Ecological Archives* A019-053-A4).

APPENDIX E

Comparison of linear, quadratic, and piecewise regressions of chlorophyll content of elk forage grasses on Julian day in 2005 (*Ecological Archives* A019-053-A5).

APPENDIX F

Temporal trends in aboveground net primary production, 2003–2006, as estimated by satellite imagery with the normalized difference vegetation index and fecal chlorophyll from piecewise regressions over the winter–spring transition for 2004, 2005, and 2006 (*Ecological Archives* A019-053-A6).

APPENDIX G

Comparison of linear, quadratic, and piecewise regressions of NDVI at fecal collection points onto Julian day of fecal collection over three winter–spring transitions (*Ecological Archives* A019-053-A7).

APPENDIX H

Comparison of linear, quadratic, and piecewise regressions of fecal chlorophyll onto NDVI at the time of fecal collection (*Ecological Archives* A019-053-A8).