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THE INFLUENCE OF DIET COMPOSITION, PLANT DEFENSIVE CHEMICALS, AND WINTER SEVERITY ON THE NUTRITIONAL CONDITION OF A FREERANGING, GENERALIST HERBIVORE

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THE INFLUENCE OF DIET COMPOSITION, PLANT DEFENSIVE CHEMICALS, AND WINTER SEVERITY ON THE NUTRITIONAL CONDITION OF A FREE-RANGING, GENERALIST HERBIVORE

By

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PREFACE

This thesis is the basis for a manuscript to be submitted to Oikos, co-authored by Jennifer S. Forbey, Brecken Robb, Rolf O. Peterson, Leah M. Vucetich, and John A. Vucetich, produced as a collaborative effort. I, Grace Parikh, performed the microhistological analysis and wrote the paper, with assistance with several collaborators, Jennifer S. Forbey performed the glucuronic acid assays of snow-urine samples and contributed to writing the manuscript. Brecken Robb assisted with the glucuronic acid assays. Rolf O. Peterson assisted with writing and editing the paper, in addition to assisting with field sample collection and obtaining funding. Leah M. Vucetich assisted with laboratory analyses. John A. Vucetich assisted with statistical analysis and writing and editing of the manuscript, as well as collection of physical samples.

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The material in Chapter 1 of this thesis is the basis for a manuscript that will be submitted for publication in *Oikos* and co-authored by J. S. Forbey (Boise State University), B. Robb (BSU), J. Vucetich (MTU), R. Peterson (MTU), and L. Vucetich (MTU). This research was supported in part by the US National Science Foundation (DEB-0918247 to J.A. Vucetich and DEB-1146194 and IOS-1258217 to J.S. Forbey), the National Institutes of Health Idaho INBRE Program (P20 GM103408), Isle Royale National Park (CESU Task Agreement No. P11AC90808), the Robbins chair in Sustainable Management of the Environment for ROP at MTU, and a McIntyre-Stennis Grant (USDANifa #224870 and USDA-Nifa#1004363).

ABSTRACT

Herbivory requires animals to manage intake of toxic phytochemicals. Detoxification and excretion of these chemicals prevents toxicity, but is energetically expensive. I investigated the relationship between investment in detoxification and nutritional condition for moose on Isle Royale National Park (*Alces alces)* during winter, using urinary indices from urine samples collected in snow. The ratio of urinary urea nitrogen:creatinine is an indicator of nutritional condition, and the ratio of glucuronic acid:creatinine is an indicator of investment in detoxification. Nutritional condition declined with greater investment in detoxification. An alternative means of managing defensive chemical intake is to diversify the diet. Microhistological analysis of fecal pellets determined diet composition. Diet diversity was weakly associated with improved nutritional condition. However, the strongest predictors of nutritional condition were winter severity and proportion of balsam fir in the diet (a dominant food for moose in this ecosystem).

CHAPTER 1

THE INFLUENCE OF DIET COMPOSITION, PLANT DEFENSIVE CHEMICALS, AND WINTER SEVERITY ON THE NUTRITIONAL CONDITION OF A FREE-RANGING, GENERALIST HERBIVORE

INTRODUCTION

Many plant secondary metabolites (PSMs), such as phenolics and terpenes (Servello and Schneider 2000), deter herbivory by being toxic in one way or another to an herbivore (Freeland and Janzen 1974, Provenza et al. 2003). For instance, PSM ingestion can inhibit enzymatic activity (Forbey et al. 2011, m et al. in press) and negatively impact nutrient absorption, energy budgets and reproductive success (Sorensen et al. 2005, DeGabriel et al. 2009, Au et al. 2013). In response, herbivores have developed various physiological mechanisms to metabolize and thereby detoxify and excrete such metabolites (Freeland and Janzen 1974, McLean et al. 2006, Sorensen et al. 2006) (Appendix 1). For that reason, one might expect that metabolism of PSMs would improve the nutritional condition of an herbivore. However, the metabolism of PSMs is energetically costly (McLean 2001, Mangione et al. 2004). If sufficiently costly, then increased investment in the metabolism of PSMs would lead to worse nutritional condition. We are unaware of any prior research to distinguish these two possibilities.

Among mammalian herbivores, the amount of glucuronic acid (GA) excreted is a useful indicator of an organism's investment to metabolize PSMs (Marsh et al. 2006b). Conjugation of PSMs with glucuronic acid is one metabolic pathway that converts PSMs

The material contained in this chapter will be submitted for publication to *Oikos*.

into water-soluble compounds, which is a prerequisite for being excreted in the urine (Villalba et al. 2005). Glucuronic acid excretion is positively associated with greater intake of specific PSMs (Guglielmo et al. 1996), as well as whole plants (Sorensen et al. 2005). This process incurs a metabolic cost of endogenous glucose (Villalba et al. 2005, Sorensen et al. 2005).

If the concentration of GA is positively associated with nutritional condition, it may indicate a net benefit whereby the nutritional benefits of detoxification outweigh the metabolic costs of detoxification. However, the concentration of GA could more simply be indicative of an herbivore that has been eating a particularly toxic diet; and while the investment in metabolizing PSMs is necessary it is also associated with poor nutritional condition resulting from a poor diet. In this paper, we assessed the relationship between GA excretion and nutritional condition for a population of free-ranging moose (*Alces alces*) (Appendix 2) during the winter. The significance of conducting this study during the winter is that at this time of year, forage is low in energy and high in toxic PSMs (Shipley et al.1998, Servello and Schneider 2000). We also conducted this assessment in two different winters, one of which was average and the other was severe (i.e., deep snow). During severe winters, the energetic cost of locomotion is greater, which is likely to influence foraging decisions (Parker et al. 1984) and may affect investment in metabolism of toxic PSMs (Sorensen et al. 2005).

Aside from metabolizing ingested PSMs, a polyphagous herbivore can also manage toxic PSMs by consuming a diverse diet. A diverse diet may minimize the rate at which any particular toxic PSM is ingested. Because many detoxification pathways are

rate-limited (Casarett et al. 2008) and PSMs are detoxified by different enzymes, a chemically diverse diet reduces the risk of saturation of an individual detoxification pathway (Freeland and Janzen 1974, Marsh et al. 2006b). That diverse diets are higher quality diets is supported by both theoretical (Westoby 1974, Marsh et al. 2006b) and empirical evidence (Bernays et al. 1994, Marsh et al. 2006a, Coltrane and Barboza 2010). Diverse diets also provide the best composition of carbohydrates, protein and micronutrients for many herbivores (Westoby 1974, Seccombe-Hett and Turkington 2008, Wang et al. 2010) including moose (Oldemeyer 1977). During summer, captive moose often consume less preferred species, even when preferred species are provided *ad libitum* (Miquelle and Jordan 1979). Free-ranging moose also feed on multiple species during summer, even when it is possible to obtain all energetic needs from one species (Miquelle and Jordan 1979). These ideas and observations suggest that diet diversity would be positively associated with nutritional condition.

However, diet diversity is likely to be advantageous only up to a point. For example, too much diversity may lead to a diet with suboptimal proportions of energy and protein (Wang et al. 2010). Additionally, ingesting a diverse diet may, under some conditions, involve increased cost associated with the longer foraging time required to encounter rare forage items and pass up common forage items. That increased foraging time could result in a reduction in overall intake rate or reduction in intake rate per unit effort spent foraging. Either case could result in reduced nutritional condition.

Most of what is known about the relationship between diet diversity and nutrition has been derived from organisms raised under relatively benign conditions, i.e. captive-

raised and/or raised on summer forage (e.g., Seccombe-Hett and Turkington 2008, Wang et al. 2010; but see Coltrane and Barboza 2010). For the first time to our knowledge, we assess this relationship in a free-ranging mammalian herbivore in a less benign environment.

STUDY SYSTEM

Isle Royale National Park is a remote island (544 km^2) located in the northwest portion of Lake Superior, North America (47º50'N, 89º00'W). The island is inhabited by a population of moose known to be influenced by predation, climate, and forage quantity (Vucetich et al. 2002, Wilmers et al. 2004, Vucetich and Peterson 2014). During the study period, moose density was between 1.7 km^{-2} and 2.1 km^{-2}. Those densities are high compared to many North American sites, but near the long-term average for this particular site.

The climate is characterized by warm summers and cold, snowy winters. For the two winters of this study, one was typical and the other was severe with respect to snow depth. In particular, the mean snow depth during the winter of 2012-13 was 29.6 cm, which represents the 13th percentile of snow depths for the period 1971-2014. During the winter of 2013-14, mean snow depth was 72.4 cm (98th percentile). Both winters were associated with lower than average predation risk (Vucetich and Peterson 2014). Both winters were associated with greater food quantity (i.e., larger bite sizes due to better twig growth) than has been typical of recent years (Vucetich and Peterson 2014).

The dominant winter food forage for this population is (in order of importance) balsam fir (*Abies balsamea*), a variety of deciduous trees and shrubs, especially American mountain ash (*Sorbus americana*), red osier dogwood (*Cornus stolonifera*), and paper birch (*Betula papyrifera)*, and cedar (*Thuja occidentalis*) (Risenhoover 1987). White pine (*Pinus strobus*), which is rare on Isle Royale, represents a very small portion of moose diet.

The eastern and western regions of Isle Royale are distinguished by important differences in vegetative composition and herbivory (Brandner et al. 1990). Key differences include the western region being characterized by increased relative abundance of cedar (Sanders and Grochowski 2012), greater browsing damage to balsam fir (Brandner et al. 1990), and smaller bite size of balsam fir (Fig. 19 in Vucetich and Peterson 2014). These differences are less likely attributable to differences in moose density, which is similar in both regions (Montgomery et al. 2013); and more likely due to differences in soil (DelGiudice et al. 1997, De Jager et al. 2009) and glacial history (Brandner et al. 1990).

METHODS

Indices of nutritional condition and investment in PSM metabolism. – To assess the nutritional condition of individual moose, we used the ratio of urinary urea nitrogen to creatinine (UN:C). That ratio is thought to be especially well suited for non-invasive assessment of nutritional condition in free-ranging animals when the purpose of assessment is to compare the condition of individuals of the same population that are

consuming similar diets (DelGiudice 1995). In mid to late winter, a moose's fat stores are often depleted, leading to negative energy balance and increased catabolism of endogenous protein stores. This is reflected by increased excretion of urinary urea at this time. Because the urine samples are collected from the snow (see below), the concentration of UN in a urine sample is influenced by the amount of snow (and the degree to which an individual is dehydrated). To account for those sources of variation, the measured concentration of UN is standardized by dividing it by the concentration of C, which is excreted at a constant rate over time (DelGiudice et al. 1991, Servello and Schneider 2000). By similar reasoning, we used the ratio, GA:C, as an index of the investment that an individual has made in metabolizing PSMs. Although GA is one of several metabolic pathways (Casarett et al. 2008), GA is positively associated with greater intake of PSMs in many taxa of herbivores (small mammals, ungulates, and birds) that were held in captivity (Guglielmo et al. 1996, Servello and Schneider 2000, Mangione et al. 2004, Sorensen et al. 2005, Sauvé and Côté 2006), thus it is a useful indicator of energetic investment by herbivores in detoxification of PSMs.

Field methods. – During January-February of 2013 and 2014, we found and followed the tracks of moose in the snow. We sampled from two different geographic regions of Isle Royale (Fig. 1). We collected pellet samples and snow-urine samples from the tracks of individual moose. We collected 34 samples in each of the two field seasons. Urine was collected as a handful of yellow snow (~6 cm^3) into a resealable plastic bag. Tracks from

which we collected samples were sufficiently spaced such that most samples represent different individuals (Appendix 3).

Laboratory methods. – Each snow-urine sample was melted and the liquid was poured into a 15 mL Falcon tube. The sample was then refrozen and stored until the time of laboratory analysis. Concentrations of UN and C were obtained by Wolff Laboratories (Minneapolis, MN), using protocols described in DelGiudice et al. (1987). We determined concentration of GA using a colorimetric assay, using protocols adapted from Blumenkrantz and Asboe-Hansen (1973).

To assess diet composition and diversity, we conducted microhistological analyses of fecal pellet samples (Holechek and Gross 1982). The process began with the preparation of the pellet samples. In particular, samples were dried and then ground by placing them in a food processor. The sample was then passed through two sieves (1 mm and 0.2 mm), rinsed with tap water, and then drained. Afterward, we incubated the sample for five minutes with 5 mL nitric acid to bleach the sample. The sample was agitated three times during the incubation period. Next, we poured the sample into a flask with 45 mL distilled water and rinsed the incubation tube with 45 mL distilled water. Then we brought the mixture of 90 mL distilled water, nitric acid, and sample to a boil for five minutes. After allowing the sample to cool, we decanted it and placed the processed sample in a vial. Using forceps and a probe, we spread a small amount of processed sample on a microscope slide and allowed it to dry for 24 hours. Afterward, we

applied three to four drops of Permount® (Fisher Scientific, Fairlawn, New Jersey), covered the sample with a 18x18 mm coverslip, and allowed it to dry for 24 hours.

We viewed the samples at 40x magnification, using polarized light. We identified the plant fragments located closest to the center of the field of view for 100 stations per slide. These stations were arranged in a grid, 10 columns and 10 rows, across the slide. Diet composition and diversity was calculated directly from the identification of these 100 fragments.

We identified each plant fragment on the basis of the structure of stomata and other cells (Appendix 4). Identifying structures were determined from a reference collection that we prepared, representing the plant species that Isle Royale moose are known to eat (Risenhoover 1987). These reference samples were ground and processed in the same manner as moose pellet samples. Because many deciduous species are difficult to distinguish, we pooled all deciduous species into one category.

STATISTICAL ANALYSIS AND RESULTS

On average, balsam fir was the most abundant food item and cedar was the least frequent of the common food items (Fig. 2). White pine represented only a trace of the diet. We observed considerable variation in diet composition (Fig. 3A). A significant portion of that variation was attributable to region (Fig. 2, $p<10^{-5}$, two-sample equality of proportions test). Mean composition did not differ significantly between the two years ($p = 0.82$).

We assessed a set of multiple linear regression models to explain variation in UN:C. The candidate predictors were GA:C; *Fir*, which is the proportion of diet that is balsam fir (Fir is both the most dominant and most variable component of the diet, see below.) and *Evenness*, which is the Shannon evenness index of diet diversity. We calculated *Evenness* as $E=H/\ln(S)$, where S is species richness, $H = -(\sum p_i \times \ln(p_i))$, and p_i is the proportion of diet comprised of one of the four food types, i (Keylock 2005). In addition to those predictors, we also consider *Year* (2013 and 2014) and *Region* (east and west) as candidate predictors. *Year* is important because the two years differed greatly with respect to winter severity. Although we do not know the precise mechanisms, previous research indicates that *Region* may also be important (see Study System). We also considered *Cedar*, but due to varied regional abundance, we omitted it from analysis (Appendix 5). We judged model performance on the basis of p values, R^2, and Akaike's Information Criterion (AIC) (Appendix 6).

More specifically, we assessed every univariate model and every model to result from all three protocols (forward, backward, and both) of R's stepwise regression procedure. Those protocols resulted in 14 models. Table 1 reports the best model from those protocols that include two, three, four and five predictors (see also Appendix 7). Because the two categorical variables were so important (see below), we also assessed every bivariate model that contained one of the categorical variables (*Year* or *Region*) and one of the continuous variables (GA:C, *Fir*, or *Evenness*).

The single most important predictor was *Year*, which explained 39% of the variation in UN:C ($p<10^{-3}$). Specifically, mean UN:C was 54% greater in 2014 than in

2013. The second most important predictor was *Fir*, which explained 23% of variation in UN:C ($p<10^{-3}$). UN:C tended to increase with more fir in the diet (Fig. 3A). The third most important predictor was GA:C (Fig.3B), which explained 11% of the variation in UN:C ($p< 0.001$). And, the fourth most important predictor was *Evenness*. After *Year* is taken into account, *Evenness* explains 7% of the variation in UN:C (e.g., model 9 in table 1). These results are, in general, supported by both univariate models and the multivariate models (Table 1). After Year, Fir, Evenness, and GA:C were considered, region did not have a statistically significant influence on UN:C (see models 13 and 14, Table 1). Variance inflation factors were low (<1.9) for the multivariate models, indicating that multicollinearity was not a concern.

The above described inferences could conceivably be statistical artifacts if diet composition estimates are overly affected by differential digestibility of food items. For example, in vitro digestibility trials indicate that balsam fir is 36% digestible, cedar is 42% digestible, and the deciduous items in this diet are 26% ($\pm$ 2.2) digestible (Fig. 4) (Risenhoover 1987). To address this concern, we repeated the analysis described in Table 1, where diet composition was adjusted to account for differential digestibility. The results of that analysis are qualitatively identical and quantitatively very similar (Appendix 8).

We repeated this procedure to explain variation in GA:C (Appendix 9) and found that the only important predictor was UN:C (Fig. 3B). In particular, GA:C is not associated with proportion of fir in the diet (Fig. 5), or evenness ($p=0.84$). Also, GA:C did not differ between years ($p=0.14$), or regions ($p=0.35$).

DISCUSSION

Greater investment in detoxification was associated with poor nutritional condition for moose during both the average winter and the severe winter (Fig. 3B). That association is consistent with the idea that detoxifying larger quantities of toxic PSMs is energetically costly (Sorensen et al. 2005) and can impair the nutritional condition of an individual (Villalba et al. 2005). Moreover, this result does not support the idea that increased investment in detoxification is associated with improved nutritional condition. In other words, high energy intake, which could plausibly allow for greater investment in detoxification, does not seem to offset the cost of detoxifying PSMs (Reid et al. 2011).

Moose with more diverse diets tended to be better nourished, in both the severe winter and the average winter (Fig. 3C). This result is similar to previous research showing improved nutrient intake and better growth with a diverse diet (Bernays et al. 1994, Dearing et al. 2000, Nersesian et al. 2012). Due to interspecific variation in the nutritional content of forage species, generalist herbivores require a variety of forage species to fulfill their nutritional requirements (Nersesian et al. 2012). Diverse diets also minimize the concentration of individual PSMs (Freeland and Janzen 1974, Provenza et al. 2003). For these reasons, diet diversity is beneficial for a generalist herbivore. Finally, the observed pattern (Fig. 3C) appears consistent with the idea that a diverse diet *may* increase search time, but not to the point of worsening an animal's nutritional condition (Wang et al. 2010).

Nutritional condition also depended on diet composition. In particular, UN:C increased with proportion of fir in the diet (Fig. 3A). This result is, at least superficially,

counterintuitive because of the two dominant food items in moose diets (balsam fir and deciduous species), balsam fir is higher quality with respect to greater digestibility, concentration of kilocalories and protein, as well as lower cellulose than the average deciduous species on which Isle Royale moose feed (Fig. 4). Bite size is also greater for balsam fir than deciduous (Table 9 in Risenhoover 1987). Bite size is an important predictor of intake rate for browsing ungulates (Shipley et al. 2007), including moose on Isle Royale (Fig. 165 in Renecker and Schwartz 1997).

The decline in nutritional condition associated with increased proportions of fir in the diet may be due to high concentrations of particularly toxic PSMs (Terra-Berns 1993, Servello and Schneider 2000). Although that inference is inconsistent with the lack of association between GA:C and fir (Fig. 5), the inference is plausible because diet composition may not be a good indicator of intake rate. Moreover, this inference may be attributed to the detoxification limitation hypothesis, which postulates that high concentrations of PSMs which limit an herbivore's capacity to detoxify PSMs will limit food intake (Freeland and Janzen 1974). For example, captive white-tailed deer fed large amounts of balsam fir substantially reduced food intake, leading to substantial loss of body mass (Ullrey 1968, Servello and Schneider 2000). It is possible that free-ranging moose eating larger proportions of fir experience nutritional restriction as a result of consuming less forage overall, not because forage is difficult to find, but due to high concentrations of toxic PSMs.

GA:C was unrelated to diet composition (Fig. 5). Diet composition is not necessarily indicative of absolute intake rate or intake rate of a particular forage item. It is

relevant to distinguish diet composition from intake rate, because GA:C has been shown to be associated with intake rate of forage species with higher concentrations of toxic PSMs (Servello and Schneider 2000). PSM concentration varies intraspecifically among individual plants, which could be an important source of unexplained variation in GA:C (Frye et al. 2013, Ulappa et al. 2014). For example, among balsam fir trees sampled on Isle Royale, the total concentration of fifteen different kinds of terpene varied by nearly a factor of three (range = [$2.3x10^3$ ppm, $6.5x10^3$ ppm]; Terra-Berns 1993).. That intraspecific variation in PSM concentration may lead to preference of some individual plants over others could be an important element of herbivory.

In addition to ecological relationships, the patterns addressed here are also due to co-evolutionary relationships between plants and herbivores. As such, our results should be considered knowing that balsam fir and northern white-cedar evolved in eastern North America, while moose evolved in Eurasia and did not encounter either species in North America until approximately fourteen thousand years ago (Hundertmark et al. 2003). Additionally, white-tailed deer, which did evolve in North America, avoid balsam fir outside of extreme conditions, but readily consume cedar (Sauvé and Cŏté 2007). These connections highlight that although many metabolic pathways for detoxification of PSMs are generic (e.g. applicable to a broad class of PSMs), there is still a great deal of unknown about the chemical ecology of herbivory.

While our original motivation was to assess the influence of investment in detoxification, diet diversity and diet composition on UN:C, the most important influence on UN:C was winter severity. Winter severity had more influence on nutritional

condition than diet composition, diet diversity, or investment in detoxification (Fig. 3). That interannual differences in UN:C account for a substantial portion (39%) of variation in UN:C (Table 1) is a necessary prerequisite for concluding that temporal variation in nutritional condition has an important influence on population dynamics.

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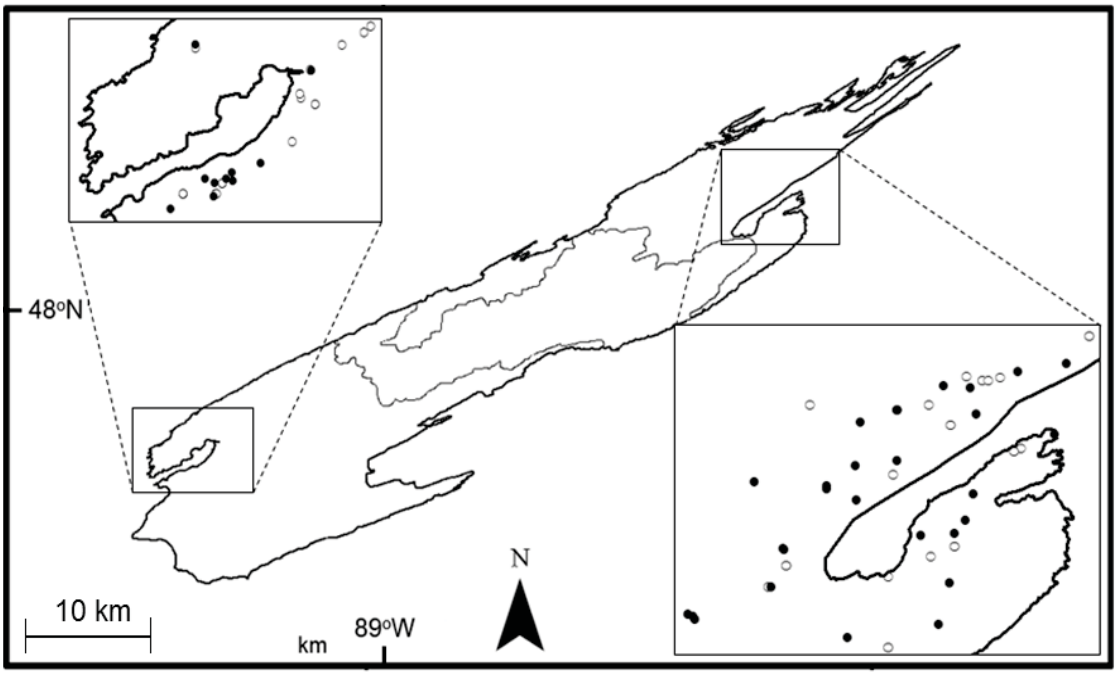

Figure 1. Distribution of sites where urine and fecal samples were collected from free-ranging moose in Isle Royale National Park in 2013 (open circles; n=34), and 2014 (filled circle; n=34). The eastern and western regions of Isle Royale are separated by the boundaries of a historic forest fire in the central portion of the island.

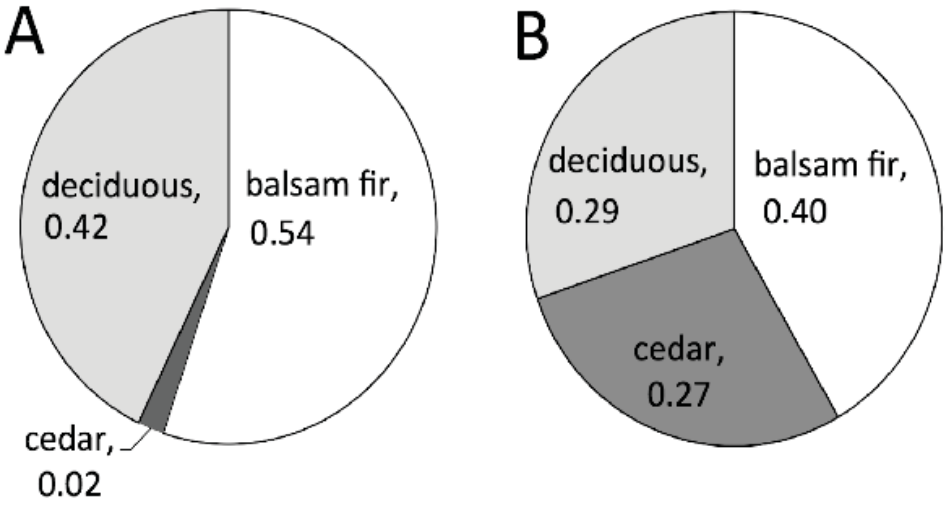

Figure 2. Composition of winter diet for moose living in the eastern (A, n = 47) and western (B, n = 21) regions of Isle Royale National Park. White pine is not depicted because it represents less than one half of one percent of diet.

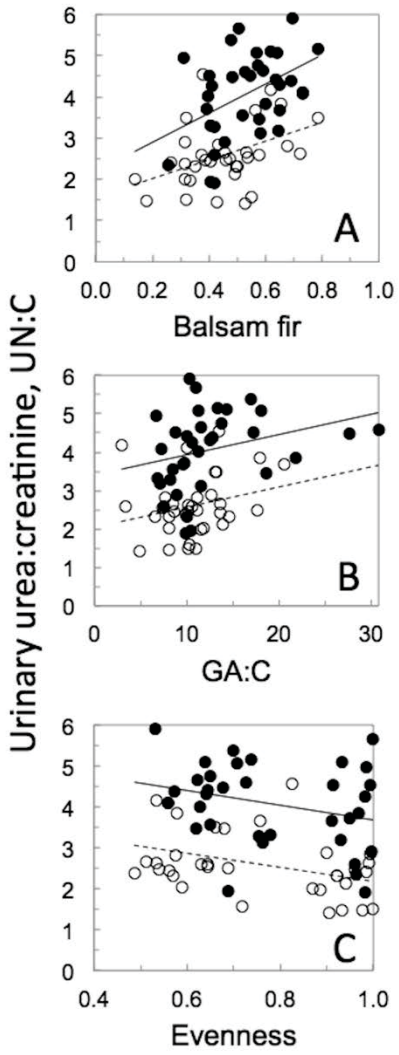

Figure 3. The ratio of urinary urea to creatinine (UN:C) and its associations with proportion of balsam fir in the diet (A, R^2=0.51, p<10^{-3}), ratio of urinary glucuronic acid to creatinine (GA:C) (B, R^2=0.44 p<0.01) and Shannon evenness index of diet diversity (C, R^2=0.46, p<0.01) for moose in Isle Royale National Park in 2013 (open symbols) and 2014 (closed symbols). The differences between years are significant in each case (p<10^{-3}, see model 7, 9, and 11 in Table 1). For context, values of UN:C greater than 3.5 are indicative of nutritional restriction in ungulates (DelGiudice et al. 1995).

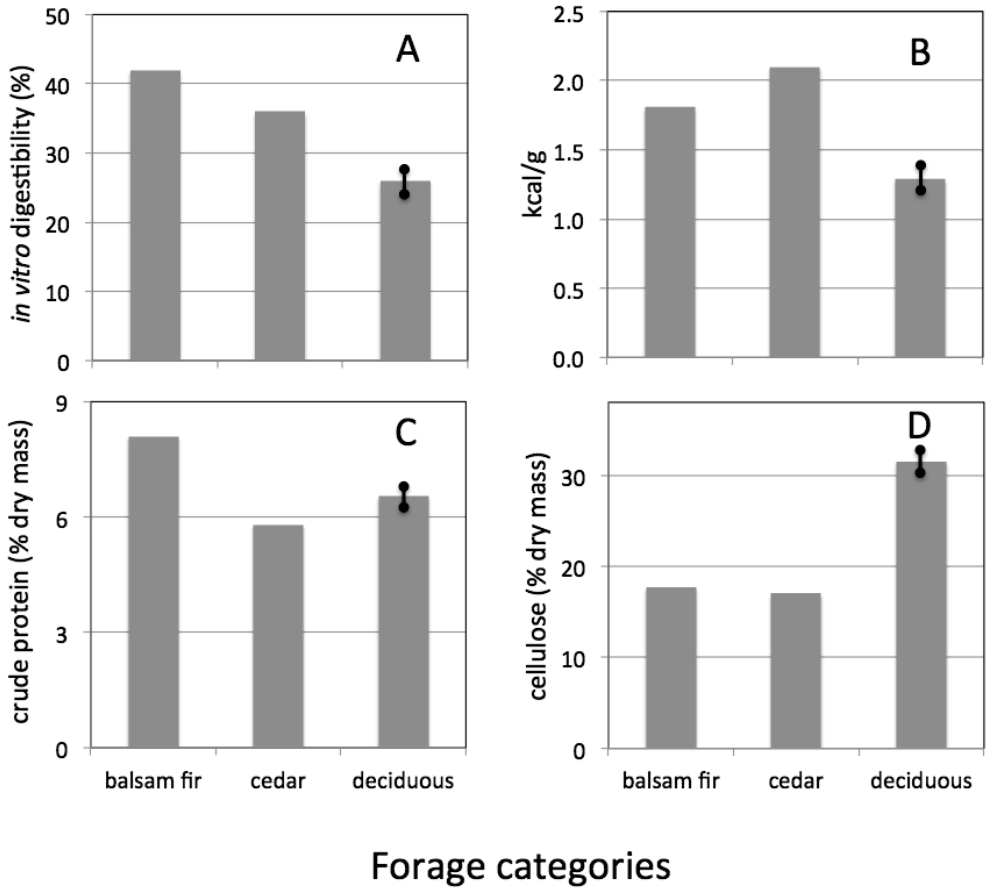

Figure 4. Digestibility (A), Energy content (B), protein content (C), and cellulose content (D) for the common forage categories of forage for moose on Isle Royale National Park during the winter. The deciduous category is an average of 15 different species that are common in the diet. The vertical bars represent standard errors. The data were taken from Appendix III of Risenhoover (1987).

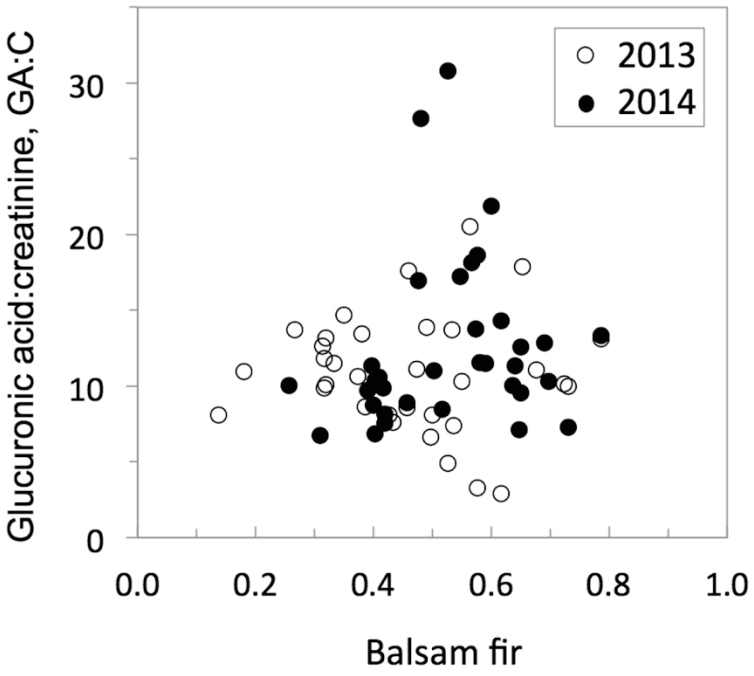

Figure 5. The ratio of urinary glucuronic acid to creatinine in relationship to the proportion of balsam fir in the diet in moose on Isle Royale National Park for two different winters. There is no significant trend (p=0.14) and no significant difference between years (p=0.14, t-test on log-transformed data).

Table 1. Performance of linear models whose response variable is the ratio of urinary urea to creatinine (UN:C) for moose living in Isle Royale National Park. The candidate predictors are the proportion of diet that is balsam fir (Fir); Shannon evenness index of diet diversity (*Evenness*), ratio of urinary glucuronic acid to creatinine (GA:C), *Region* (east or west, see Fig. 1), and *Year* (2013 or 2014). Values in parentheses are p-values. See *Statistical Analysis and Results* for additional details.

Model	Predictor(s)	R^2	dAIC
1	Fir ($<10^{-3}$)	0.23	31.4
2	Evenness (0.14)	0.03	46.6
3	GA:C (<0.01)	0.11	41.2
4	Region (<0.01)	0.12	40.1
5	Year ($<10^{-3}$)	0.39	14.7
6	Fir (<0.01), Region (0.19)	0.25	31.9
7	Fir ($<10^{-3}$), Year ($<10^{-3}$)	0.51	3.3
8	Evenness (0.81), Region (0.01)	0.12	42.3
9	Evenness (<0.01), Year ($<10^{-3}$)	0.46	9.4
10	GA:C (0.01), Region (<0.01)	0.20	35.7
11	GA:C (0.02), Year ($<10^{-3}$)	0.44	11.6
12	GA:C (0.03), Fir ($<10^{-3}$), Year ($<10^{-3}$)	0.54	0.31
13	Fir (<0.01), Evenness (0.11), GA:C (0.03), Year ($<10^{-3}$)	0.56	0.0
14	Fir (<0.01), Evenness (0.27), GA:C (0.03), Region (0.68), Year ($<10^{-3}$)	0.56	2.3

APPENDIX 1-Plant Secondary Metabolites-Toxicity and Metabolism

Polyphagous herbivores feed on a wide range of plants to meet nutritional needs, while balancing intake of potentially toxic plant secondary metabolites (PSMs). Because plant secondary metabolites are difficult to avoid completely, herbivores have developed an array of mitigation mechanisms, ranging from limiting consumption of particularly toxic PSMs to metabolism of ingested PSMs (Fig. A1).

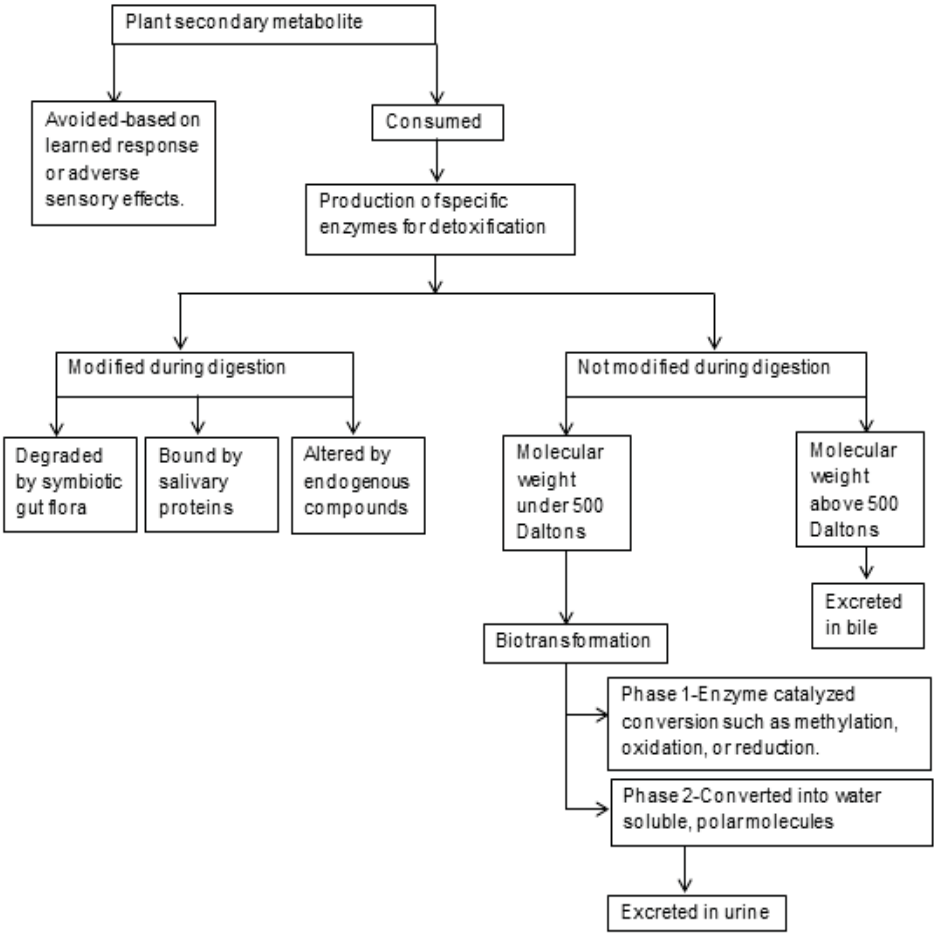

Figure A1. Mechanisms used by herbivores to manage PSMs in forage. An herbivore's decision to consume a particular PSM is a function of detoxification capacity and availability of alternative foods. Compounds that are ingested are detoxified in a variety of ways, allowing for a chemically diverse diet. Adapted with permission from Marsh et al. (2006b)[1].

If a PSM is particularly toxic, tolerance is low, leading to avoidance. This behavior may be a learned response or due to adverse sensory properties of the plant (e.g. foul taste or smell that deters consumption) (Marsh et al 2006b).

If a PSM can be metabolized, the animal will consume it at subtoxic levels and excrete it, thereby preventing toxic accumulation. Detoxification mechanisms for PSMs are very diverse, allowing polyphagous herbivores to consume a diverse diet. Some mechanisms act prior to digestion (e.g. by gut microbes or salivary binding proteins) (Marsh et al 2006b). Many PSMs are absorbed in the gut for metabolism and excretion.

Of the PSMs absorbed in the gut, most are small enough to be excreted in the urine after biotransformation. Biotransformation occurs in two phases, which may be sequential or simultaneous. Phase 1 reactions (functionalization) are enzyme-catalyzed conversions, including oxidation, hydrolysis, methylation and reduction. Most of these reactions are catalyzed by cytochrome P450 enzymes (Dearing et al. 2005). In Phase 2 (conjugation), compounds are converted into water-soluble, polar compounds that are readily excreted. Many pathways exist in both phases to cope with a chemically diverse diet. Because individual PSMs require specific enzymes for metabolism, it is possible for multiple compounds to be detoxified by a particular reaction (Marsh et al. 2006b).

Duration of detoxification is a function of concentration and intake of a specific PSM. A specific pathway may become saturated due to depletion of an enzyme or cofactor, leading to toxic accumulation of a PSM (Marsh et al. 2006b).

Although PSMs are often toxic, many have beneficial properties which are exploited by animals (Forbey et al. 2009). For example, animals sometimes seek out PSMs for antiparasitic effects (Forbey et al. 2009, Forbey and Hunter 2012). Like other pharmacologically active

compounds, potency of a PSM is a function of the amount consumed, known as dose-response. Up to a certain point, a compound is inert, and even beneficial in some cases when consumed below the toxicity threshold (Fig. A2). The likelihood of using a PSM for self-medication is a function of the metabolic cost and potential benefits of ingestion (Forbey et al. 2009, Forbey and Hunter 2012). If the benefits of self-medication with a PSM outweigh the metabolic cost of ingestion, an herbivore will be more likely to consume it. If the metabolic cost exceeds the benefits of self-medication, an herbivore will be less likely to do so (Forbey et al. 2009).

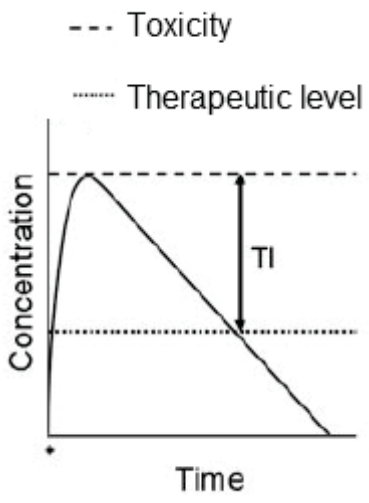

Figure A2. Hypothetical dose-response curve for a PSM. In this case, the concentration of a PSM in an animal stays below the toxicity threshold, while remaining within the therapeutic concentration limits for the maximum amount of time. Adapted with permission from Forbey et al. (2009)[2].

That polyphagous herbivores avoid toxicity by consuming a chemically diverse diet raises questions about how specialist herbivores avoid PSM toxicity on a single species diet. This is explained by different mechanisms associated with each feeding strategy.

Generalist herbivores absorb larger quantities of PSMs in their guts than their specialist counterparts, leading to greater investment in detoxification. Specialist herbivores excrete greater amount of unmetabolized PSMs (Sorensen et al. 2005). Additionally, specialist herbivores

employ compensatory feeding; increased energy intake offsets the metabolic costs of detoxification. Such a strategy is not observed in generalist herbivores (Sorensen et al. 2005).

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APPENDIX 2. Brief review of the basic ecology of moose (*Alces alces*)

Morphology

Moose are the largest member of the cervid (deer) family (Franzmann 1981, Gaillard 2007), weighing 200-825 kg (Gaillard 2007). Cervids are often distinguished by branched deciduous antlers displayed by males. These organs develop as apices of the perennial pedicles on the skull (Fig. A3), with a gradual mineralization process. All hormonal activity in the body is connected to antlerogenesis, either directly or indirectly (Bubenik 1997a).

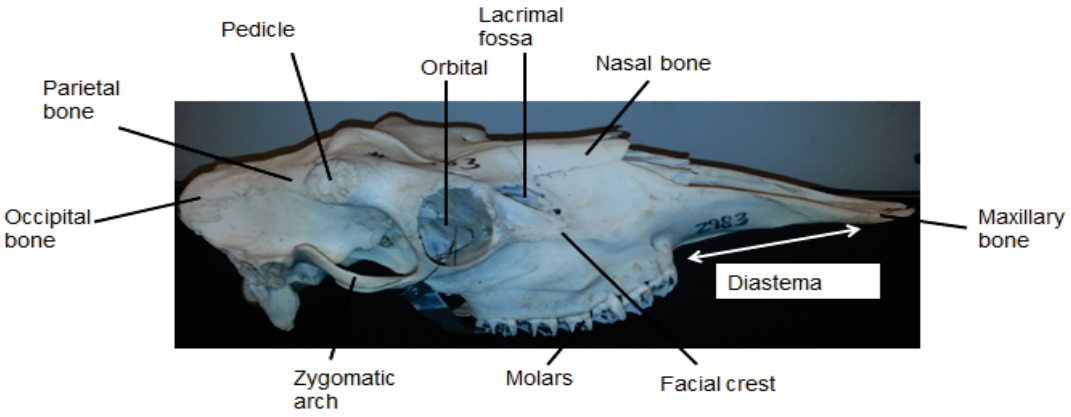

Figure A3. Moose skull and upper jaw. The facial bones comprise 70% of the skull, while the cranial bones comprise 30% (Bubenik 1997a). The upper jaw lacks incisors and canines.

The dentition of moose is consistent with other browsing herbivores, with a large oral cavity and diastema (gap between the incisors and molars and premolars) (Bubenik 1997a). While teeth are worn by biting and chewing, the physical pressure stimulates

development of protective cementum layers (Fig. A4). Summer cementum layers are generally thicker and denser than in winter (Gasaway et al. 1978, Bubenik 1997a).

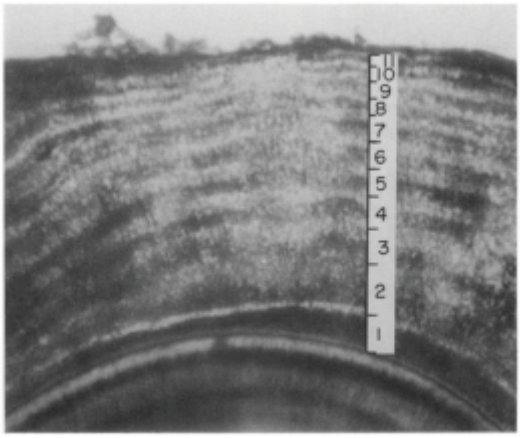

Figure A4. Cross section of an incisor from a moose of undetermined age. Opaque cementum. Reprinted with permission from Gasaway et al. (1978)[3].

In addition to distinct dentition, moose have skeletal characteristics adapted to traversing soft ground and snow. The skeletal configuration is conducive to a large stride. An animal's gait is determined in large part by the angle between the scapular spine and axis of the humerus. In a moose, this angle is approximately 140 degrees, compared to less than 120 degrees in most other ungulates. Long forelimbs enable moose to traverse deep snow or soft ground (Fig. A5).

Additionally, the phalanges of the hooves provide stability on such terrain. The dewclaws and phalanges readily spread for stability and contract when lifted to enable movement. A moose's hooves have long soles, which are keratinized over the last phalanges as well as the dewclaw (Fig. A6). In juveniles, rapid growth leads to wear of hooves. Similarly, rapid wear of the hooves occurs in rutting bulls (Bubenik 1997a).

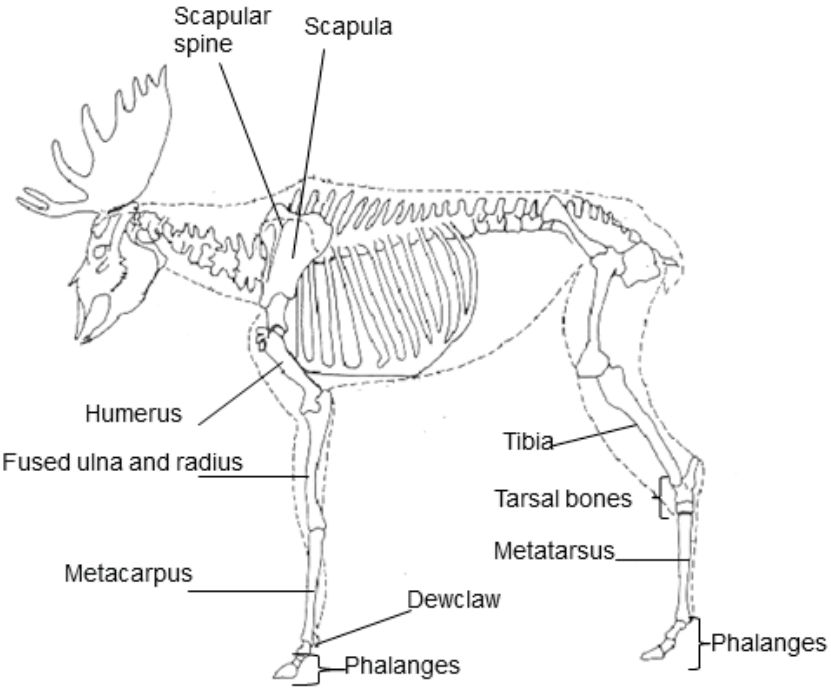

Figure A5. Skeleton of a moose. Adapted with permission from Bubenik (1997a)[4].

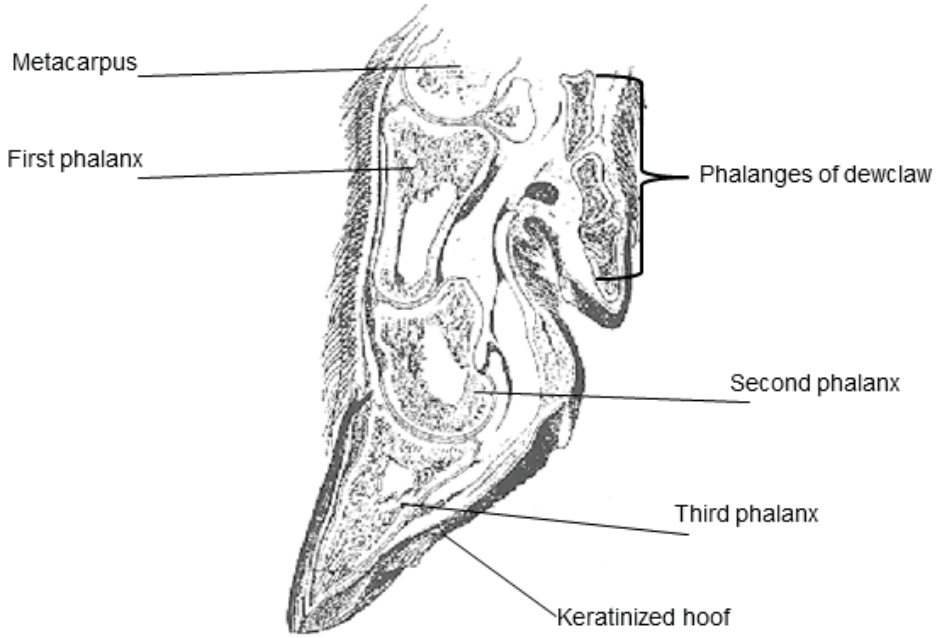

Figure A6. Anatomy of a moose's foot. Adapted with permission from Bubenik (1997a)[4].

Distribution and Habitat.

Moose inhabit mixed boreal forests throughout the northern hemisphere. A key difference between Eurasian moose habitat and North American moose habitat is the greater agricultural and forestry activity in the former. Moose are unique to the northern hemisphere, with no moose populations or analogous habitat types in the southern hemisphere (Karns 1997).

Northern distribution of moose is limited by food availability and cover, while southern distribution is limited by climate, specifically temperature. Moose are particularly vulnerable to heat stress, with upper critical temperatures of 5.1^{o}C in the winter and 14^{o}C in the summer. Lower critical temperatures are undetermined for moose (Karns 1997). Moose are able to thermoregulate by exploiting conifer forests in winter and aquatic habitat in summer (Franzmann 1981, Peek 1997).

While summer presents the risk of heat stress, winter also imposes physiological and energetic challenges on moose. The variable characteristics of snow (density, compaction, depth) can affect locomotion and food availability (Peek 1997, Renecker and Schwartz 1997). At depths of over 1 m, locomotion becomes more difficult for moose (Peek 1997). In response, moose will travel sparingly and occupy areas with less snow cover (Peek 1997).

In addition to seasonality, a moose's age, sex, and reproductive status also influence habitat choice and trade-offs. More specifically, cows with calves will select

habitat with adequate cover from predators, while males tend to select habitat to maximize energy gain, with potentially higher predation risk (Bjorneraas et al. 2012). Due to deteriorating body condition, senescent moose tend to select habitat with maximum predation coverage, at the expense of forage quality (Montgomery et al. 2013).

Feeding.

Moose, like other polyphagous herbivores, forage in such a manner that maximizes nutrient and energy gain and minimizes uptake of plant secondary metabolites. Moose require abundant, high quality browse. However, environmental conditions and PSMs can severely limit food intake.

Additionally, food intake can be limited by rumen capacity. High fiber foods slow digestion and increase rumination time. As a result, a moose may not take in enough food to fulfill energy demands. Unlike many large ruminants, moose are unable to efficiently extract energy from high fiber forage, leading to more selective feeding. As a concentrate selector, moose are less able to extract energy from fibrous forage, leading to more selective feeding than roughage feeders (e.g. bison) or intermediate feeders (e.g. elk) (Risenhoover 1987, Renecker and Schwartz 1997). Digestive morphology is significantly different between these animals, and reflecting by their feeding strategies (Fig. A7).

As browsers, moose consume large amounts of woody plants, which are higher in lignin than grass and herbs. Lignin decreases digestibility and requires extended rumination time (Risenhoover 1987, Duncan and Poppi 2008). Food remains in the rumen until nutrient extraction is maximized and fragments are sufficiently broken down

and able to pass to the omasum and abomasum (Renecker and Schwartz 1997, Duncan and Poppi 2008).

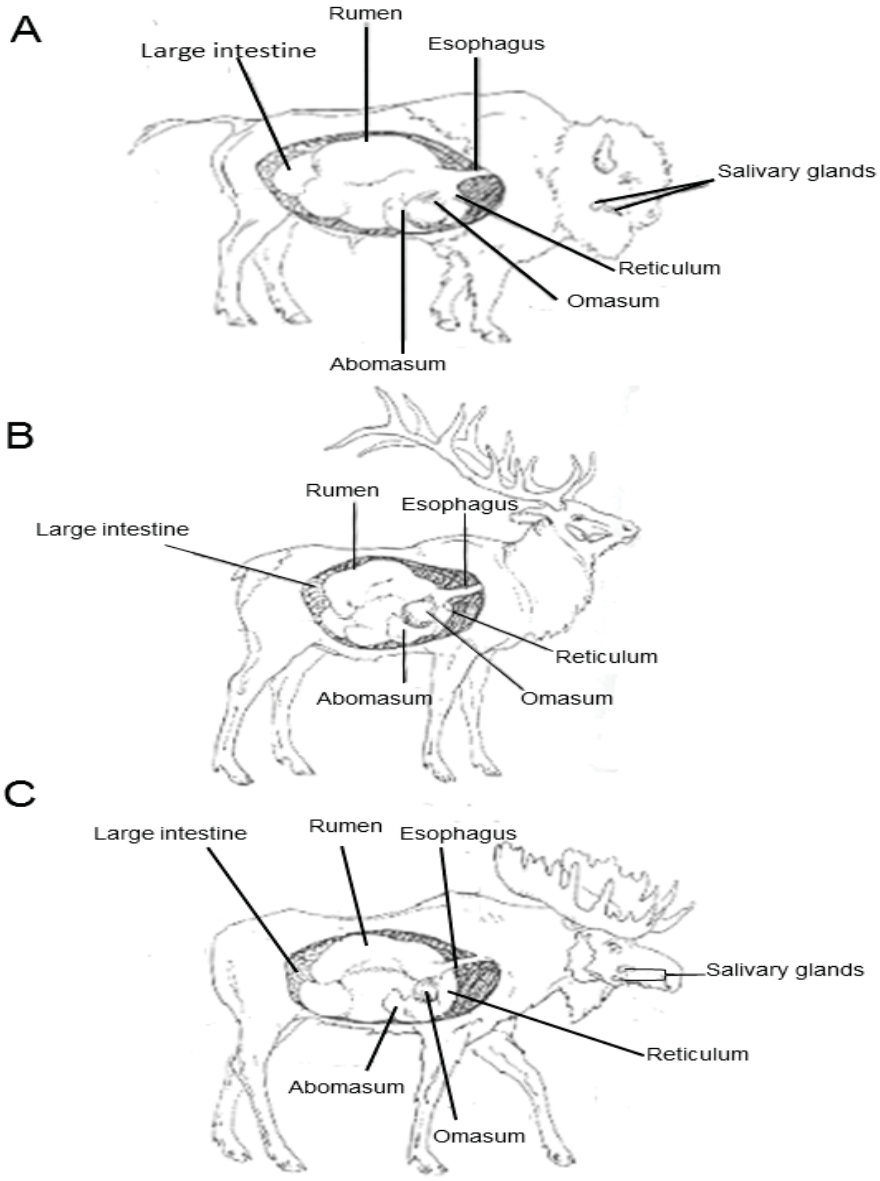

Figure A7. Comparison of digestive systems of ruminants based on feeding strategy. A)Bison-roughage feeder (primarily grass and forbs); B) Reindeer-intermediate feeder (both browse and grass/forbs); C)Moose-concentrate selector (selective, easily fermentable browse). Although all 3 feeding strategies require regurgitation and rumination of food, passage and fermentation rates differ. A roughage feeder

has a large, subdivided rumen and long intestinal tract, allowing for digestion of fibrous foods. An intermediate feeder is able to adapt rumen absorption based on season. A concentrate selector has a smaller rumen, shorter gut and rapid passage, requiring substantial salivation to buffer the rumen and slow digestion. Adapted with permission from Renecker and Schwartz (1997)[4].

Time constraints limit a moose's availability to seek out high quality forage (Risenhoover 1987). Searching for forage decreases available time to handle food items, since the two are mutually exclusive (Stephens and Krebs 1986).

For moose, foraging entails several trade-offs, such as feeding rate versus digestibility .These trade-offs occur seasonally, as well as in response to predation risk. In some cases, a moose will seek spend the minimum time foraging to fulfill basic nutritional needs. Such circumstances are more likely in winter, when all forage is similarly low quality. In other cases, when food is abundant and predation risk is lower, a moose will seek to maximize nutrient gain, spending more time foraging (Renecker and Schwartz 1997).

Foraging decisions are hierarchical, with key criteria such as quality of forage, density, and quantity in a bite. However, higher density of moose leads to less selective foraging and greater browsing damage in the area (Renecker and Schwartz 1997).

During summers, moose rely heavily on aquatic habitat, for both thermoregulation and food. Aquatic forage species are highly digestible (Karns 1997), and also contain substantial levels of sodium, an essential micronutrient (Belovsky 1981).

Seasonal fluctuations in mass are attributable to food availability and quality. A moose's metabolism appears to have an endogenous rhythm, making mass fluctuations

predictable. A male moose's mass peaks in early rut and then up to 23% of mass is lost over winter. A female moose's mass peaks in early winter, and reaches its lowest point after parturition, where up to 19% of mass is lost (Schwartz 1997).

Reproduction

Like many ungulates, reproductive rates in moose popuations are a function of nutrition and habitat quality. In most habitats, more than 70% of adult female moose ovulate in a given year, with low rates of ova loss. Most females give birth to a single calf in spring, although twinning is not uncommon (Franzmann 1981). Twinning frequency is a function of habitat quality and population in comparison to the habitat's carrying capacity (Schwartz 1997).

Estrus occurs in late summer (Fig. A8), lasting 1-2 days. During this time, bulls are attentive to receptive cows. Moose are polyestrous, which means that failure to conceive causes a cow to repeat the estrus cycle, up to 6 times in one season. Cessation of estrus depends on seasonal shifts in photoperiod (Schwartz 1997).

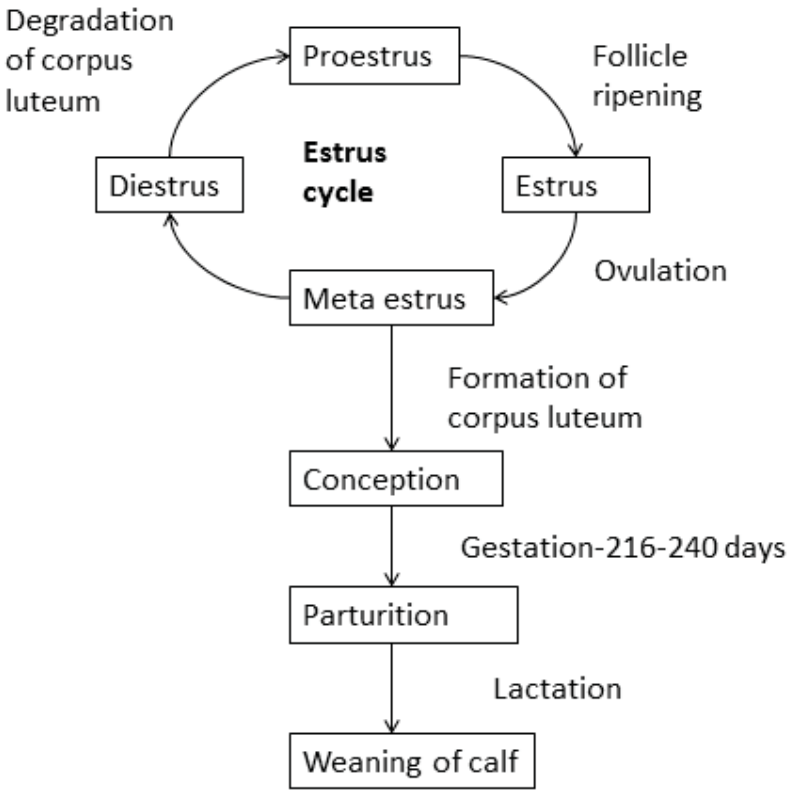

Figure A8. Annual reproductive cycle of a female moose. The estrus cycle involves development of ovarian follicles, which rupture and release eggs, which are fertilized during breeding. Within a ruptured follicle, the corpus luteum develops and releases progesterone, to stimulate fetal development. If an egg is not fertilized, the corpus luteum disintegrates and the cycle repeats. Gestation takes place during winter, and birth occurs in late May. Lactation occurs during summer, and weaning is complete by late August. Adapted from Schwartz (1997)[4].

A cow moose first ovulates between 16-28 months of age, although poor nutrition may delay ovulation by up to a year. While yearlings are sexually mature, they do not breed as consistently as their adult counterparts. In high quality range, yearling ovulation is much more common, suggesting importance as a gauge of population condition (Schwartz 1997).

Gestation occurs over winter, lasting from 216-244 days (Franzmann 1981, Schwartz 1997). Parturition generally occurs in May. Following parturition, lactation imposes a substantial energy cost on a cow moose. On average, spring mass gain for lactating cows is 12% lower than their non-lactating cohorts. For calves, early growth is a function of milk quality and quantity, with shifts in composition over time. Weaning generally occurs by 2 months of age.

Prime reproductive age is between 7 and 12 years of age for females (Franzmann 1981). Healthier females tend to begin breeding younger, up to a point. Primiparous second-year moose cows tend to have better nutritional condition than their cohorts with calves (Schwartz 1997). While females commonly reproduce as yearlings, it is far less common in males due to competition by dominant males (Schwartz 1997).

A male calf will develop pedicles by autumn, from which rudimentary antlers develop in the first year. Incomplete antlerogenesis occurs for the first 4 years. Optimum antler size and form occurs at 10 years of age. In senescent moose (between 12-14 years), antler palms narrow and reduce to a function as display organs during breeding season. Antlerogenesis begins in spring, continuing through summer when the antlers are fully developed. As testosterone production increases, antlers mineralize and velvet is shed prior to autumn rut. Active spermatogenesis also occurs. Following rut, testosterone production plummets, leading to shedding of antlers and sterility. Breeding behavior ceases until early spring, when the cycle repeats (Fig. A9).

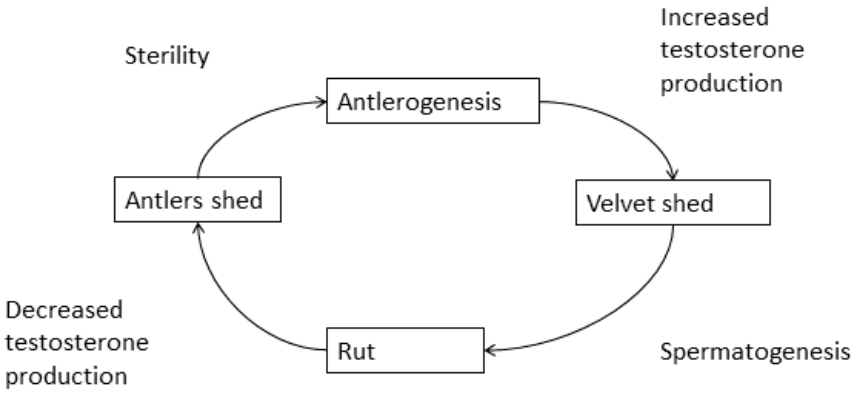

Figure A9. Annual reproductive cycle of a male moose. In early spring, testosterone production stimulates antlerogenesis. By autumn, velvet is shed and antlers are ossified. Following rut, decreased testosterone production leads to shedding of antlers and a sterile period during winter. Adapted from Schwartz (1997)[4].

Behavior

Behavioral characteristics of moose are often dictated by the demographics. Although moose are not gregarious, it would be erroneous to classify them as solitary animals. Bubenik (1997b) characterizes moose as individualistic. A moose has an individual range of variable size, and is not antagonistic towards other animals in most cases. However, a female selects and designates mating territory, where male suitors approach and are selected based on rank (assessed by antler size) (Bubenik 1997b).

Communication in moose occurs in several ways, often combining multiple means. For instance, body posture and ear positioning convey if an animal is aggressive, alert, or actively listening for other animals (Bubenik 1997b).

Vocalizations are used by different demographics as a communication method. A cow's vocal repertoire is broader than a bull's, with there is little overlap between the sexes. A calf will vocalize to seek its mother or nurse. Many moose will produce a grunt as a "greeting" or other acknowledgment. During estrus, a receptive female will produce moaning sounds, detected by males via echolocation (Bubenik 1997b).

Distressed or antagonistic calls are also produced, ranging from a snort to a loud nasal whine. Rutting bulls will often roar, and cows will often roar in the presence of a human (Bubenik 1997b).

Chemoreception is an integral communication tool used by moose. Urinary pheromones indicate an estrous cow's receptivity, and males apply salivary musk to antlers. Additionally, cows mark mating territory using olfactory cues (Bubenik 1997b). Moose have an acute sense of smell, which is bolstered by the presence of a Jacobson's (vomeronasal) organ, which detects less volatile chemicals not sensed by the nose (Bubenik 1997b).

Excluding mating females, moose are not described as territorial. However, aggression still occurs, particularly among males. Aggressive behavior is a function of testosterone levels, peaking in prime-aged males with antlers. In this case, sparring is common, particularly during rut. After antlers are cast, testosterone levels drop, leading to less fighting and aggregation of males with little contact with cows. Aggression remains minimal until testosterone rises with antler ossification (Bubenik 1997b).

A hierarchy also exists among males. When a subordinate male wishes to enter the individual zone of a dominant male, the subordinate will offer an antler to spar. The dominant male may spar or offer an antler for an olfactory check, in which case the subordinate male may stay nearby and forage (Bubenik 1997b).

Excluding distress, moose generally move with a slow gait. Moose are well-adapted to environmental conditions. For instance, moose may travel through deep snow using forelegs as snowshoes, kneeling and crawling forward, limiting deep snow to the forelegs. Moose are also powerful swimmers, often diving for submerged plants (Bubenik 1997b).

A moose's daily activity is a function of photoperiod, as well as age and reproductive status. Seasonality also determines activity. During winter time, moose may conserve energy by staying in areas of less snow accumulation (Peek 1997).

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APPENDIX 3. Evidence that most samples belong to different individual moose

To minimize the frequency of re-sampling the same moose multiple times, we abandoned trails after collecting a sample and then sought a new set of tracks to follow. Typically the next set of tracks was at least 0.4 km away.

We have been collecting samples in this manner for a number of years prior to this study. In prior years we had also analyzed microsatellite DNA extracted from fecal samples (13 microsatellite loci and sex chromosomes). From those DNA profiles we determined individual identities of each sampled moose. The results of that analysis are described in Table A1.

Table A1.The number of unique individuals was determined via the analysis of fecal DNA at 13 microsatellite loci and markers on the sex chromosomes. This determination was possible because most snow urine samples collected between 2004 and 2010 were also paired with fecal pellets that had been deposited alongside in the same snow tracks where the urine had been sampled. The average ratio among years is 0.68. That ratio is an indicator of the number of unique individuals that one can expect to have sampled, given the number of UN:C samples collected.

Year	No. of UN:C samples	No. unique individuals	Ratio of UN:C samples to unique individuals
2004	53	39	0.74
2007	54	41	0.76
2008	86	53	0.62
2009	99	69	0.70
2010	112	65	0.58

APPENDIX 4. Microhistology as a technique for determining diet composition of browsing herbivores

Microhistology determines an herbivore's diet composition by fecal analysis Forage species are identified microscopically, based on species-specific cuticle structures. Use of fecal analysis is a non-invasive, cost-effective method, but has some limitations (Anthony and Smith 1974, Fitzgerald and Waddington 1979).

Microhistology relies on the assumption that all plant cuticles survive the digestive tract, which is not necessarily true. It is necessary to be familiar with the phenology of food plants in the ecosystem of focus. Microhistology may not be applicable in all seasons, due to differential digestion, which may skew results. Not all food plants are observed in fecal matter, and results may be skewed towards plants that are detected in fecal matter. For this reason, microhistology is dependent on seasonality.

Additionally, microhistology requires substantial training of analysts. A reference collection is necessary for this purpose. In creating a reference collection, plants should be processed in the same manner as fecal pellets (Fitzgerald and Waddington 1979).

For this research, observers underwent thorough training to ensure high accuracy in analysis. Additionally, in the Isle Royale ecosystem, winter forage is less diverse than in summer. Winter forage is also higher in structural carbohydrates and diagnostic features of food plants are more visible in scat.

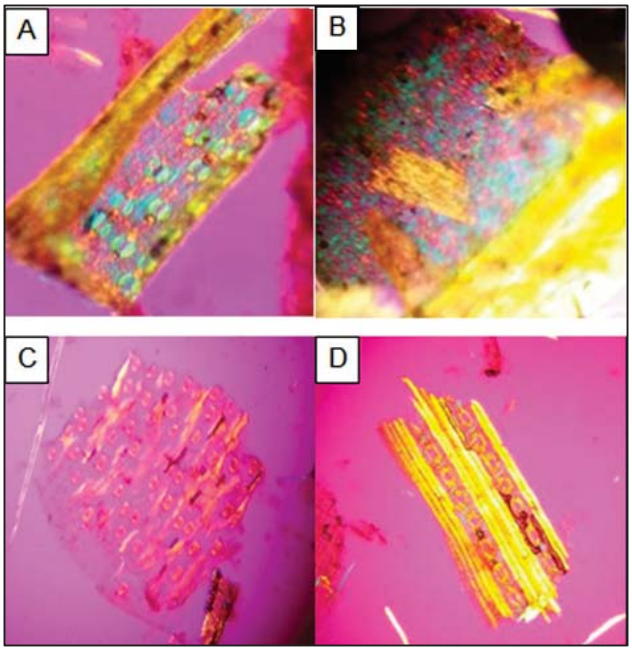

Figure A10. Photographs of microscopic fragments of food items that moose in Isle Royale National Park are known to eat during winter, using polarized light microscopy. A)balsam fir (*Abies balsamea)*, B)deciduous, C)Northern white cedar *(Thuja occidentalis)*, D)white pine (*Pinus strobus)*.

During winter on Isle Royale, moose feed on conifer leaves and deciduous twigs. These two groups of species can be distinguished by the presence of stomata (structures regulating gas exchange [Alberts 2004]) in conifer leaves. Each species has a distinct stomata structure (Fig. A10). Deciduous twigs lack stomata, and instead are characterized by fibrous fragments and blocky cell structure, and species are not readily distinguished. For this project, we used polarized light microscopy due to improved contrast and visibility of diagnostic features of plant fragments.

References for Appendix 4

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APPENDIX 5. Influence of cedar on urea nitrogen:creatinine

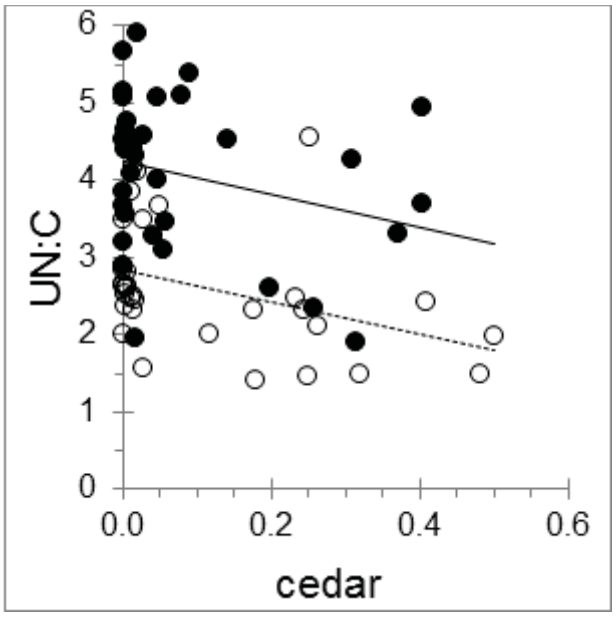

Figure A11. Relationship between urinary urea nitrogen:creatinine and proportion of cedar in the diet in moose on Isle Royale National Park. Samples from 2013 are denoted by open circles, and samples from 2014 are denoted by solid circles.

Unlike balsam fir, greater proportions of cedar in the diet improved nutritional condition for moose (Fig. A11). Energy content is similar between the two species, although cedar is more digestible than fir (Risenhoover 1987). Coniferous species contain particularly toxic PSMs (Servello and Schneider 2000), which may counteract energy gain from consumption. Our results are consistent with previous research on captive white-tailed deer. When fed cedar-based diets, deer exhibited lower mass loss and greater food intake than when fed fir-based diets (Servello and Schneider 2000). That deer ate less when fed balsam fir is consistent with the detoxification limitation hypothesis, which states that an organism's food intake is limited by capacity to detoxify specific PSMs (Freeland and Janzen 1974). That deer ate more when fed cedar suggests

that cedar may contain smaller amounts of toxic PSMs that may limit food intake (Freeland and Janzen 1974).

Because cedar is abundant only in the western region (Sanders and Grochowski 2012), we omitted it from analysis (Table A2). In the eastern region, cedar comprised a very small portion of the diet due to limited availability.

Table A2. Models assessing urinary urea:creatinine. The candidate predictors are the proportion of diet that is balsam fir (*Fir*); the proportion of the diet that is cedar (Cedar); Shannon evenness index of diet diversity (*Evenness*), ratio of urinary glucuronic acid to creatinine (GA:C), *Region* (east and west, see Fig. 1), and *Year* (2013 and 2014). Values in parentheses are p-values. See *Statistical Analysis and Results* for additional details.

Model	Predictors	R^2	dAIC
1	Cedar (0.01)	0.09	41.24
2	Cedar (0.13), Region (0.93)	0.12	40.85
3	Cedar (<0.01), Year ($<10^{-3}$)	0.46	8.00
4	GA:C (0.01), Cedar (0.04), Year ($<10^{-3}$)	0.49	5.63
5	Cedar (0.24), Evenness (0.12), GA:C (0.04), Year ($<10^{-3}$)	0.51	5.29
6	Fir (<0.01), Cedar (0.84), Evenness (0.89), GA:C (0.01)	0.30	29.38
7	Fir (0.01), Cedar (0.33), Evenness (0.79), GA:C (0.01), Region (0.13)	0.33	28.77
8	Fir (0.01), Cedar (0.94), Even (0.18), GA:C (0.03), Year ($<10^{-3}$)	0.56	0.00
9	Cedar (0.66), Evenness (0.18), GA:C (0.04), Region (0.66), Year ($<10^{-3}$)	0.51	7.42

10	Fir (0.01), Cedar (0.75), Evenness (0.26), GA:C (0.03), Region (0.61), Year ($<10^{-3}$)	0.56	2.04

APPENDIX 6. Background information pertaining to the statistical methods

Models were evaluated by using a combination of AIC, R^2, and p-values. Additionally, multicollinearity was assessed using variance inflation factors.

R^2 is known as the coefficient of determination. This statistic expresses the proportion of variation in a response variable that is explained by a set of predictors in a given model. For a multivariate model, R^2 signifies the square of the coefficient of multiple correlations. This value is computed by the formula

$$R^2 = 1 - \frac{R_{ss}}{SS_{tot}}$$

where R_{ss} is the residual sum of squares (discrepancy between model estimation and actual data) and SS_{tot} is the total sum of squares (sum of squared differences between each observation and the mean of the data set) (Burnham and Anderson 2002).

In addition to assessing explanatory power, it is also necessary to assess the statistical significance of a set of models, often using p-values. A p-value indicates the probability of obtaining a result equal to or more extreme than the observed sample when the null hypothesis is true. A p-value is compared to a predetermined value α (usually 0.05). If the p-value is smaller than α, there is strong evidence against the null hypothesis. If the p-value exceeds α, there is little to no evidence against the null hypothesis (Burnham and Anderson 2002). Both the R^2 and p-value were calculated using the lm() function in R.

Akaike's Information Criterion (AIC) is a measurement of quality of a single model within a set of models. A lower AIC value indicates a better model. This value

concerns the trade-off of model fit versus complexity. A penalty (increased AIC) is incurred for a model with a higher number of predictors, which curtails the risk of overfitting a model. AIC is computed by the formula AIC=-2k – 2ln(L) where k is the number of parameters in the model, and L is the log of the maximum likelihood of the model. This value can also be calculated in R using the AIC() command. For a small sample size, a corrected AIC should be calculated using the formula $AIC_c = AIC + \frac{2k(k+1)}{n-k-1}$, where k is the number of parameters in the model and n is the sample size. Models are evaluated based dAIC, which is difference in AIC from the best model of a set (which has a dAIC of 0). A dAIC of less than 2 indicates a better model.

Although AIC is useful in selecting models, it is not useful for assessing model quality in absolute terms. If all models in a set are poor in an absolute sense (e.g. large p-values), AIC will still select the best model of the set (Burnham and Anderson 2002).

In multivariate models, multicollinearity is a concern. In a given model, the severity of multicollinearity is determined by variance inflation factors, which determine the magnitude of inflation of the standard error of a parameter caused by multicollinearity. This value is calculated using the mass package in R, and the command VIF(). A VIF of 5 would indicate that the standard error of a parameter is larger by a factor of 5 than the value calculated without considering intercorrelation of predictors. Most sources state that a VIF of 10 or greater indicates severe multicollinearity.

References for Appendix 6

Burnham, K. P. and Anderson, D. A. 2002. Model Selection and Multimodel Interference. – Springer-Verlag.

APPENDIX 7. Performance of regression models predicting UN:C.

Table A3.Performance of regression models predicting the ratio of urinary urea to creatinine (UN:C). This table is an extension of Table 1. It includes every model that resulted from all three versions of the *step* command in R (forward, backward, and both). The dAICc values are calculated in relationship to model 13 in Table 1. The candidate predictors are the proportion of diet that is balsam fir (*Fir*); Shannon evenness index of diet diversity (*Evenness*), ratio of urinary glucuronic acid to creatinine (GA:C), *Region* (east and west, see Fig. 1), and *Year* (2013 and 2014). Values in parentheses are p-values. See *Statistical Analysis and Results* for additional details.

Model	Predictors	R^2	dAIC
15	Fir ($<10^{-3}$)	0.23	31.42
16	Fir ($<10^{-3}$), Evenness (0.82)	0.23	33.62
17	Fir ($<10^{-3}$), GA:C (0.01)	0.30	26.74
18	Fir ($<10^{-3}$), Year ($<10^{-3}$)	0.50	3.26
19	Fir (<0.01), Region (0.19)	0.25	31.86
20	GA:C (0.01), Region (<0.01)	0.20	35.72
21	Fir (<0.01), GA:C (0.01), Region (0.24)	0.32	27.59
22	Fir ($<10^{-3}$), GA:C (0.03), Year (<0.001)	0.54	0.31
23	Fir ($<10^{-3}$), Evenness (0.79), GA:C (0.01)	0.30	28.99
24	Fir (<0.01), Evenness (0.60), Region (0.17)	0.25	33.89
25	Fir (<0.01), Evenness (0.11), Year ($<10^{-3}$)	0.52	2.91
26	Evenness (0.91), GA:C (0.01), Region (0.02)	0.20	38.03

27	Fir (<0.01), Region (0.19), Year ($<10^{-3}$)	0.52	3.77
28	Fir (<0.01), Evenness (0.69), GA:C (0.01), Region (0.22)	0.32	29.82
29	Fir (<0.01), Evenness (0.11), GA:C (0.03), Year ($<10^{-3}$)	0.56	0.00
30	Fir (<0.01), GA:C (0.03), Region(0.23), Year ($<10^{-3}$)	0.55	1.16
31	Fir (<0.01), Evenness (0.27), GA:C (0.03), Region (0.68), Year ($<10^{-3}$)	0.56	2.29

APPENDIX 8. Accounting for differential digestibility and its influence on model results

The forage species vary with respect to digestibility. In particular, cedar was 42.1% digestible, balsam fir was 36.2% digestible, and deciduous species were 26.2% (±2.2%, standard error for 15 species) digestible (Appendix III of Risenhoover 1987). We did not have an estimate of digestibility for white pine. However, it represents a very small portion of diet (i.e., <0.5%). Moreover, because the digestibility of conifers was greater than that of the deciduous species, the best estimate of digestibility that is available to us is the average digestibility of the other two conifer species (i.e., 39.2%).

To verify that our results and conclusions were not influenced by differences in digestibility, we repeated the analysis represented in Table 1, except that estimated diet composition was replaced with estimated that were adjusted for differences in digestibility. The adjusted proportion of diet for food category *i* was:

$$\frac{p_i/d_i}{\sum_i(p_i/d_i)}$$

where p_i is the unadjusted proportion and di is digestibility of food category *i.* The regression analysis reported in Table 1 is presented again in the table below (on the next page), except that proportion of diet that is fir and evenness were recalculated with adjusted proportions.

We also performed equality of proportions tests to assess a quantitative difference between raw diet composition and adjusted diet composition values. We compared raw proportions and adjusted diet proportions within each region and found no significant difference between raw and adjusted diet proportions (East, p=0.77; West, p=0.67). We

repeated this test comparing diet proportions within each year, and found no significant difference (2013, p=0.77; 2014, p=0.73). These analyses suggest that diet composition was essentially unaffected by adjustment for digestibility.

To verify that *Evenness* was not affected by adjustment for digestibility, we used a paired t-test to compare evenness values between raw diet proportions and adjusted diet proportions. There was no significant difference between adjusted and raw evenness values (p=0.53), indicating that diet diversity indices were not affected by this adjustment.

Table A4. Performance of regression models predicting the ratio of urinary urea to creatinine (UN:C) for moose living in Isle Royale National Park, where diet composition is adjusted for differential digestibility of the forage items. The candidate predictors the proportion of diet that is balsam fir (*Fir*); Shannon evenness index of diet diversity (*Evenness*), ratio of urinary glucuronic acid to creatinine (GA:C), *Region* (east or west, see Fig. 1), and *Year* (2013 or 2014). Values in parentheses are p-values. See *Statistical Analysis and Results* for additional details.

Model	Predictors	R^2	dAIC
1	Fir ($<10^{-3}$)	0.20	33.55
2	Evenness (0.37)	0.01	47.76
3	GA:C (<0.01)	0.11	41.02
4	Region (<0.01)	0.12	39.89
5	Year ($<10^{-3}$)	0.39	14.49
6	Fir (<0.01), Region (0.07)	0.24	32.29
7	Fir ($<10^{-3}$), Year ($<10^{-3}$)	0.50	3.81
8	Evenness (0.39), Region (<0.01)	0.13	41.38
9	Evenness (0.04), Year ($<10^{-3}$)	0.43	12.47
10	GA:C (0.01), Region (<0.01)	0.20	35.50
11	GA:C (0.02), Year ($<10^{-3}$)	0.44	11.34
12	Fir ($<10^{-3}$), GA:C (0.03), Year ($<10^{-3}$)	0.54	0.82
13	Fir ($<10^{-3}$), Evenness (0.09), GA:C (0.03), Year (<0.001)	0.56	0.00
14	Fir (<0.01), Evenness (0.33), GA:C (0.03), Region (0.46), Year ($<10^{-3}$)	0.56	1.89

APPENDIX 9: Performance of regression models predicting the ratio of glucuronic acid to creatinine (GA:C) for moose living in Isle Royale National Park.

Table A5. Performance of regression models predicting the ratio of urinary glucuronic acid to creatinine (GA:C) for moose living in Isle Royale National Park. The models listed include every model that resulted from all three versions of the step command in R (forward, backward, and both). The candidate predictors are the proportion of diet that is balsam fir (*Fir*); Shannon evenness index of diet diversity (*Evenness*), *Region* (east or west, see Fig. 1), and *Year* (2013 or 2014). Values in parentheses are p-values. See *Statistical Analysis and Results* for additional details.

Model	Predictors	R^2	dAIC
1	Fir (0.37)	0.01	1.41
2	Evenness (0.84)	$<10^{-3}$	2.20
3	Region (0.35)	0.01	1.32
4	Year (0.15)	0.03	0.00
5	Fir (0.39), Evenness (0.93)	0.01	3.67
6	Fir (0.61), Region (0.55)	0.02	3.30
7	Fir (0.58), Year (0.20)	0.04	1.93
8	Region (0.47) , Year (0.18)	0.04	1.70
9	Fir (0.66), Evenness (0.86), Year (0.20)	0.04	3.97
10	Evenness (0.93), Region (0.53), Year (0.21)	0.04	4.03
11	Fir (0.79), Region (0.60), Year (0.21)	0.04	3.97
12	Fir (0.78), Evenness (0.90), Region (0.61), Year (0.25)	0.04	6.36

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1. Appendix 1, Figure A1

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Original figure numbers	Figure 1
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2 messages

Grace Parikh <glparikh@mtu.edu> Wed, Mar 18, 2015 at 12:47 PM
To: karen.marsh@anu.edu.au

Hello Dr. Ford,

I am a finishing graduate student at Michigan Technological University.

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The figure was adapted from the manuscript

Marsh, K. J. et al. 2006b. The detoxification limitation hypothesis: where did it come from and where is it going? – J. Chem. Ecol. 32:1247-1266.

I have attached the adapted version. I look forward to your reply.

Regards,

Grace Parikh

Karen Ford <karen.marsh@anu.edu.au> Wed, Mar 18, 2015 at 5:36 PM

To: Grace Parikh <glparikh@mtu.edu>

Hi Grace,

Not a problem. You are welcome to use the figure.

Cheers,

Karen.

Karen Ford

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2. Appendix 1. Figure A2.

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Publisher of your work	n/a
Expected publication date	Apr 2015
Permissions cost	0.00 USD
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3. Appendix 2, Figure A4

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2 messages

Grace Parikh <glparikh@mtu.edu> Wed, Feb 4, 2015 at 12:40 PM
To: Permissions@wiley.com

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Grace Parikh

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4. Appendix 2, Figures A5-A9

E&M of North American Moose

4 messages

Steve Williams <swilliams@wildlifemgt.org> Wed, Feb 4, 2015 at 2:32 PM
To: "glparikh@mtu.edu" <glparikh@mtu.edu>

Grace
Would you please provide the adapted images so I can see what has changed. I don't suspect this will be a problem but I would need to review first. Thank you.

Steve Williams
President
Wildlife Management Institute
717.677.4480 (o)
717.677.4233 (f)
swilliams@wildlifemgt.org

Grace Parikh <glparikh@mtu.edu> Wed, Feb 4, 2015 at 2:51 PM
To: Steve Williams <swilliams@wildlifemgt.org>

Hello Steve.

Thank you for your prompt response.

I have attached a file with the adapted images, as well as notes of locations of the original figures.

I look forward to your reply.

Regards,

Grace Parikh

Steve Williams <swilliams@wildlifemgt.org> Fri, Feb 6, 2015 at 2:34 PM
To: Grace Parikh <glparikh@mtu.edu>

Grace
You have WMI's permission to use the adapted images with the footnotes that you have indicated on the examples. Best of luck.

Steve Williams
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