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The Nutritional Condition of Moose Co-Varies with Climate, but Not with Density, Predation Risk, or Diet Composition

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Abstract

A fundamental question about the ecology of herbivore populations pertains to the relative influence of biotic and abiotic processes on nutritional condition. Nutritional condition is influenced in important, yet poorly understood, ways by plant secondary metabolites (PSMs) which can adversely affect a herbivore's physiology and energetics. Here we assess the relative influence of various abiotic (weather) and biotic (intraspecific competition, predation risk and diet composition) factors on indicators of nutritional condition and the energetic costs of detoxifying PSMs for the moose population in Isle Royale National Park (U.S.A.). Specifically, we observed interannual variation in the ratio of urea nitrogen to creatinine (UN:C), an indicator of nutritional restriction, over 29 years and the ratio of glucuronic acid to creatinine (GA:C), an indicator of energetic investment, in detoxifying PSMs over 19-years. Both UN:C and GA:C were measured in samples of urine-soaked snow. Most importantly, climatic factors explained 66% of the interannual variation in UN:C, with moose being more nutritionally stressed during winters with deep snow and during winters that followed warm summers. None of the biotic factors (density, predation, diet composition) were useful predictors of UN:C or GA:C. The absence of a relationship between diet composition and either UN:C or GA:C suggests that the nutritional ecology of wild herbivores is probably complicated by fine-scale variation in protein content and concentrations of PSMs amongst plants of the same species. UN:C increased with GA:C at both the individual and population-level. That result is consistent with detoxification being energetically costly, such that it impairs nutritional condition and also highlights how spatio-temporal variation in the intake and detoxification of PSMs may influence population dynamics. Lastly, because we observed interannual variation in nutritional condition over three decades and detoxification over two decades these findings are relevant to concerns about how herbivore populations respond to climate change.

Keywords: *Alces alces*, chemical ecology, diet, detoxification pathway, foraging ecology, forage quality, glucuronic acid, Isle Royale National Park, nutritional ecology, plant secondary metabolites, protein, urea, vertebrate herbivore

Introduction

A perennial interest in ecology is to understand the relative influence of biotic (density dependence and predation) and abiotic (weather) factors on population dynamics (Coulson et al., 2001; Vucetich and Peterson, 2004). Of particular importance is to improve understanding of the relative influences of biotic and abiotic factors on nutritional condition, because nutritional condition is an important determinant of reproduction and survival for many vertebrate species (Parker et al., 2009). Understanding how nutritional condition fluctuates in response to weather, and factors such as population density, predation risk and foraging behaviors is also relevant for understanding the influence of climate change on animal populations. However, new insights on these topics are limited by the difficulty of simultaneously measuring nutrition-related phenomena (e.g., indices of body condition), biotic factors and abiotic factors for free-ranging vertebrate populations over sufficiently long periods of time and large enough spatial scales.

For herbivores, the influence of abiotic and biotic processes on nutritional condition are complicated by the physiological consequences of consuming plant secondary metabolites (PSMs). Some PSMs can inhibit digestion, metabolism, and nutrient assimilation (Sorensen et al., 2005b; Au et al., 2013; Kohl et al., 2015). Because of those negative effects, herbivores may manage their intake of PSMs by altering behaviors, such as habitat selection, diet selection and food intake rates (Torregrossa and Dearing, 2009; Frye et al., 2013; Ulappa et al., 2014). Herbivores can also mitigate the effects of ingesting PSMs by detoxifying them, for example, via conjugation with glucuronic acid (GA, (Servello and Schneider, 2000)). However, the process of detoxifying PSMs is thought to involve both energetic costs (involving the loss of glucose) and protein costs (Guglielmo et al., 1996; Sorensen et al., 2005b; Au et al., 2013).

The influence of PSMs on nutritional condition and feeding behavior for vertebrate herbivores have mostly been evaluated using feeding trials on captive animals (Guglielmo et al., 1996; Sorensen et al., 2005b; Au et al., 2013). Directly assessing a herbivore's total intake of PSMs is challenging for free-ranging vertebrate populations because it requires measuring the volume of each food type consumed and chemical analysis of browsed foliage, which is especially difficult given that concentrations of PSMs vary substantially among plant species, among plants of the same species and among parts of the same plant (Sauvé and Côté, 2007; Frye et al., 2013; Ulappa et al., 2014). However, captive studies typically involve relatively small sample sizes, and artificial diets that have substantially higher concentrations of particular PSMs and less diverse mixtures of PSMs than herbivores would encounter in the wild. The few studies that have evaluated the relationship between PSMs and nutritional condition for free-ranging vertebrate populations typically span only 1-3 years and focus on variation among individuals. However, there is emerging evidence that the costs associated with ingesting, absorbing and metabolizing PSMs could influence reproductive performance (DeGabriel et al., 2009) and ultimately population dynamics (DeAngelis et al., 2015). Consequently, there is value in assessing long-term fluctuations in PSM consumption and the concomitant energetic costs in free-ranging herbivores at the population-level.

In this study, we investigated interannual fluctuations in two nutritional indicators and assessed how they varied with abiotic and biotic covariates and with each other for a free-ranging population of moose in Isle Royale National Park (IRNP). More precisely, we used samples of urine-soaked snow collected during winter to observe interannual fluctuations in the ratio of urea nitrogen to creatinine (UN:C), an indicator of nutritional condition (DelGiudice, 1995). We also observed interannual fluctuations in the ratio of glucuronic acid to creatinine (GA:C), an indicator of energetic investment in detoxifying PSMs that have been ingested and absorbed by vertebrate herbivores (Parikh et al. 2017, Guglielmo *et al.* 1996; Sorensen *et al.* 2005, Servello & Schneider 2000). We assessed the relative influence of abiotic and biotic covariates on UN:C over a 29-year period and on GA:C over a 19-year period (for details see *Nutritional indicators*). The abiotic variables were seasonal temperatures and precipitation. The biotic covariates were moose population density, predation risk and two indices of diet composition (the proportion of deciduous forage in the diet and diet diversity). We also assessed relationships among these variables for moose living in two regions of on Isle Royale (eastern and western region). Previous research suggests that these regions differ with respect to the relative abundance of forage types (Sanders and Grochowski, 2011), diet composition and the physiology of moose (mean levels of UN:C and GA:C, Parikh et al. 2017, DelGiudice *et al.* 1997), but do not differ in terms of moose density and predation risk (see *Study system*).

Our assessment of spatio-temporal variation in these two nutritional indicators was partly guided by the preceding background and a set of five specific hypotheses:

1. Nutritional restriction and investment in detoxification would be greater following warmer summers and during winters with deeper snow. Those weather conditions are expected to increase metabolic and energetic costs, cause moose to reduce food intake rates, and seek shelter in habitats with dense canopy cover where the abundance and quality of forage is lower (Moen, 1976; Parker, Robbins, & Hanley, 1984; Shively, Crouse, Thompson, & Barboza, 2019; Thompson, Crouse, Jaques, & Barboza, 2020; van Beest & Milner, 2013). Moreover, warmer temperatures have been shown to influence the PSM profiles of at least some plant species that moose forage on in this region (Berini et al., 2018). (For additional details see *Abiotic and biotic predictors.*)
2. Nutritional restriction and investment in detoxification would be greater in years when moose abundance and predation risk were higher. Higher herbivore densities can lead to increased competition and food restriction by reducing both the quantity and quality of forage available (DeAngelis et al. 2015). Additionally, habitat selection and movement are influenced by density and predation risk for many ungulate populations (Fortin et al., 2005; van Beest et al., 2016). Predation risk can also affect herbivores' selectivity for certain forage types within a given site (Hoy et al., 2019) and can influence diet quality (Barnier et al., 2014). Thus, if high moose density or greater predation risk result in moose consuming lower quality diets (e.g., consuming plants with more PSMs) then those effects of moose density and predation risk on diet quality may also affect how much moose invest in detoxification or reduce their nutritional condition.
3. Nutritional restriction would be greater in years when moose invested more in detoxifying PSMs. Prior research indicates that the detoxification of PSMs can be energetically costly (Sorensen et al. 2005; Au et al. 2013) to the point of impairing nutritional condition for herbivores in captive environments with controlled diets (Villalba, Provenza & Bryant 2002). Moreover, GA:C and UN:C were found to be positively correlated for a sample of free-ranging individuals (total of 68 individual herbivores sampled over a 2-year period, Parikh et al. 2017). Although those assessments focused on *individual*-level variation, our assessment goes further by assessing whether interannual variation in nutritional restriction co-varies with investment in detoxification at the *population*-level, in two regions, and over timescales that are relevant to population ecology.
4. Investment in detoxifying PSMs will be lower during winters when moose consume more deciduous forage or have less diverse diets. That hypothesis is based on the observation that captive ungulates tended to invest less in detoxification when their diets were dominated by deciduous forage (Servello and Schneider 2000) and the idea that herbivores may minimize their intake of specific PSMs by maintaining a diverse diet (Freeland and Janzen, 1974; Dearing et al., 2000; Marsh et al., 2006).
5. Nutritional restriction may be greater during winters when moose consume more deciduous forage because deciduous forage contains more cellulose, is less digestible, and sometimes contains less protein than some coniferous species in winter (see Fig. 4 in Parikh et al. 2017). Alternatively, it is plausible that if herbivores invest less in detoxifying PSMs when they consume deciduous forage it may compensate for the disadvantages of deciduous forage being less digestible and containing less protein.

Materials & Methods

Study System

Isle Royale National Park (544 km^2) is located in Lake Superior, North America (47°50′N, 89°00′W). The climate in IRNP is temperate, and characterized by short, warm summers (July-September) and long, snowy winters (with snow cover typically starting at the end of October and lasting until April). See Supporting Information S1 for additional details about interannual variation in weather. The moose population in IRNP has been studied continuously since 1959. Moose are the only large herbivores on Isle Royale, but beaver (*Castor canadensis*) influence some parts of the forest, mostly aspen (*Populus tremuloides*) close to lakes and streams (Moen, Pastor, & Cohen, 1990). The moose population is not hunted, and grey wolves (*Canis lupus*) are the only predator. Wolf predation has a strong influence on the dynamics of this moose population (Vucetich et al., 2011; Peterson et al., 2014). Predation and moose abundance have varied widely over the 29-year study period (see Fig. S1).

On Isle Royale, balsam fir (*Abies balsamea*) is the most abundant and most used winter forage species for moose (McLaren and Peterson, 1995). Balsam fir typically represents 47% of winter diet, northern-white cedar (*Thuja occidentalis*) represents 15%, and the remainder is comprised of a variety of deciduous species (Risenhoover, 1987;

Parikh et al., 2017). Cervids are thought to prefer balsam fir over other coniferous species (e.g., *Picea glauca*) because it contains lower concentrations of condensed tannins (Sauvé and Côté, 2007), which bind with protein and make protein less available to herbivores (McArt et al., 2009; Barbehenn and Constabel, 2011). However, concentrations of PSMs can vary considerably among plants of the same species. For example, the concentration of 15 different PSMs varied by nearly a factor of three in balsam fir samples collected from the study site (Terra-Berns, 1993).

Diet composition differs between the eastern and western regions of this study site, with moose in the east consuming more balsam fir and substantially less cedar. Moose in the western region also tend to have more diverse diets in terms of species evenness (Parikh et al., 2017). These regional differences in diet are likely due to spatial variability in the diversity and quality of available forage. For example, compared to the eastern region, the western region is characterized by a higher relative abundance of cedar and more diverse deciduous woody browse species (DelGiudice et al., 1991; Sanders and Grochowski, 2011). The protein content of balsam fir is also generally higher in the western region than in the east (Fig. S2 in Supporting Information S2). The eastern and western regions of the study site are separated by a 35 to 50 km wide region of low-quality winter habitat that tends not to be used by moose during winter (Montgomery et al., 2014). However, moose densities are similar and temporally correlated in the eastern and western region (typically, 1.4 to 2.4 moose/km^2 (Montgomery et al., 2014)).

Nutritional Indicators

Concentrations of urinary metabolites provide useful information about an individual's health and physiological processes. For example, the ratio of urea nitrogen to creatinine (UN:C) in urine-soaked snow is a useful indicator of the nutritional condition of ungulates during mid-late winter (DelGiudice, 1995). Urea is the largest component of nitrogen in urine, and UN production is directly related to the catabolism of dietary and endogenous proteins (Barboza et al., 2020). During periods of low protein intake, such as mid-late winter, mammals often increase catabolism of endogenous protein, resulting in increased concentrations of UN in urine. Estimates of UN obtained from snow-urine samples can be adjusted to correct for differences in dilution associated with the snow, the herbivore's level of hydration and body size by measuring the concentration of creatinine (C) in the urine (DelGiudice, 1995). Creatinine results from creatine phosphate metabolism in skeletal muscles which is excreted as a waste product exclusively in urine at a relatively constant daily rate that is proportional to the individuals lean muscle mass (DelGiudice, 1995). Consequently, concentrations of C in snow-urine serve as a baseline that facilitates comparisons among individuals (DelGiudice, 1995). High ratios of UN:C can indicate lower endogenous energy reserves, lower food intake, lower assimilation of nutrients from ingested food, greater energy expenditure, or some combination of these factors. Although UN:C can also be high when animals consume high protein diets, moose do not have access to high protein foods in winter. Thus, UN:C from urine samples collected during mid-late winter typically reflect the amount of "nutritional restriction" experienced by an individual (DelGiudice, 1995; Parikh et al., 2017). For context, in mid-late winter a UN:C > 3.5 is indicative of accelerated catabolism of endogenous protein, substantially reduced nutritional condition, and starvation in ungulates (DelGiudice, 1995).

The ratio of glucuronic acid to creatinine (GA:C) in urine-soaked snow is a useful indicator of an individual's energetic investment in detoxifying PSMs via the glucuronidation pathway (Parikh et al., 2017). The use of GA:C as an indicator of energy invested in detoxifying PSMs is justified by glucuronic acid (GA) being a known derivative of endogenous glucose. Moreover, conjugation of PSMs with GA is known to be a major detoxification pathway for vertebrate herbivores (Servello and Schneider, 2000) and feeding trials with captive birds and mammals have shown that increasing intake of PSMs is causally correlated with increased excretion of GA (Guglielmo *et al.* 1996, Sorensen et al. 2005). Therefore, although GA:C is not a direct measure of the total amount of PSMs a herbivore consumes, it is useful for reflecting energetic investment in detoxifying PSMs for free-ranging herbivores.

Collection of Field Samples

Each winter over an approximately 4-week period (mid-January to mid-February) we followed fresh tracks left by individual moose until we found a patch of urine-soaked snow and then collected approximately 350 cm^3. To minimize the frequency of re-sampling the same individual, after collecting a urine sample, we left the area and travelled approximately half a kilometer before searching for the next set of fresh tracks to follow. Genetic analysis of a subsample of moose fecal pellets, collected using the same method as urine samples, suggest that approximately 70% of the samples we collect within a given year are from unique individuals (Parikh et al. 2017).

As winter progresses, nutritional restriction might increase and lead to declines in body mass which could influence the amount of UN and C excreted in the urine. Indeed, experimental studies on captive ungulates (with controlled diets) have showed that the ratio of UN:C in snow urine samples fluctuated between late-October to mid-April (Parker et al., 2005). Therefore, it is plausible that variation in sampling dates could influence our results. However, that concern is alleviated by linear regression models suggesting that the day of the year that samples were collected explained less than 2% of the variation in concentrations of UN, C, and GA in snow-urine samples. Moreover, our sampling period did not vary substantially among years and was relatively short (4-week) compared to the 5-month period considered in the aforementioned experimental study by Parker, Barboza & Stephenson (2005).

Each year over a 13-year period (2004-2015 and 2017), we also collected 10-20 fecal pellets from pellet piles found at sites in both the eastern and western region of Isle Royale to estimate the composition of winter diet (Parikh et al., 2017; Hoy et al., 2019). In most cases, it was not possible to determine whether a pellet and snow-urine sample came from the same individual moose in a given year. Therefore, we conducted our dietary analyses at the population-level, rather than the individual-level.

In total, we assayed UN:C for 2,123 snow-urine samples (*n* = 1,068 western region; *n* = 1,055 eastern region) collected over a 29-year period (1988-2015 and 2017). We also assayed GA:C for 755 of these samples (*n* = 371, western region; *n* = 384, eastern region) collected over a 19-year period (1994, 1997, 2000-2015 and 2017). We estimated diet composition for 627 pellet samples (*n* = 344, western region; *n* = 283, eastern region) over the 13-year period (2004-2015 and 2017). See Supporting Information S3 for additional details on sample collection and sample sizes.

Laboratory Methods

We measured concentrations of UN and C using spectrophotometry at Biovet USA Inc (Burnsville, MN) by following protocols described in DelGiudice, Mech, Seal, & Karns (1987). We estimated concentrations of GA using a colorimetric assay (Parikh et al., 2017). We estimated diet composition from microhistological analysis of plant fragments from fecal pellets following the procedures described in Parikh et al. (2017). For each pellet sample, we identified 300 plant fragments on the basis of cell structures (i.e., stomata and other distinguishing features) and estimated the proportion of those plant fragments that were from balsam fir, cedar, or deciduous species (Parikh et al., 2017; Hoy et al., 2019). We also followed Parikh et al. (2017) and used this diet composition data to calculate an index of diet diversity, the evenness of the three food types (Keylock, 2005).

Statistical Analyses

Hereafter, we use $UN{:}C_t$ and $GA{:}C_t$ to denote the two response variables, mean annual UN:C and mean annual GA:C, respectively. First, we used linear models to assess the relative influence of biotic and abiotic factors on $UN{:}C_t$ and $GA{:}C_t$ for the entire population. All statistical analyses were performed in Program-R version 4.0.5 (R Core Team, 2016). We also repeated this analysis for the eastern and western regions separately because we had *a priori* reason to suspect that nutritional restriction and investment in detoxification may differ between the two regions (i.e., because of regional differences in forage availability and diet). We assessed the relationship between five abiotic (weather) variables and $UN{:}C_t$ and $GA{:}C_t$. We included mean annual snow depth (*snow*) as a variable that might influence nutritional condition and investment in detoxification because snow depth can influence energetic costs of movement (Moen, 1976; Parker et al., 1984), and winter-habitat selection for ungulates (Montgomery et al., 2013). We also included the mean North Atlantic Oscillation (*NAO*) between Dec-March because NAO is a useful index of winter severity for many ungulate populations, including moose in IRNP (Vucetich and Peterson, 2004). Prior research suggests that NAO, a large-scale atmospheric measure, may be a better indicator of winter severity than locally-measured indicators (Hallet et al., 2004; Vucetich and Peterson, 2004). In the northeastern United States, a negative NAO is typically indicative of a winter with stronger cold-air outbreaks and increased storminess, whereas a positive NAO is associated with milder, less stormy winters. We hypothesized that moose would be more nutritionally stressed and invest more in detoxification during severe winters (i.e., winters with deep snow and when NAO was negative).

We also included indices of weather conditions during the previous growing season because of their potential effects on plant growth, forage quality, and moose body condition attained during summer, which is thought to be an important determinate of body condition throughout the rest of the year for some ungulates (Cook et al., 2013). We included precipitation during the previous growing season (*precip*) and the cumulative number of growing-degree-days (*GDD*) until June 15th as candidate predictors. *GDD* indicates plant phenology and the timing of spring "green-up". Lastly, we included mean temperature during the previous summer (*temp*) because of its potential effects on the growth and PSMs profiles of important forage species (Reich et al., 2015; Berini et al., 2018), and on moose physiology (e.g., heart and respiration rates) and behavior (e.g., habitat selection and forage intake rates (van Beest and Milner, 2013; Shively et al., 2019; Thompson et al., 2020)). We hypothesized that moose would be more nutritionally stressed and invest more in detoxification following hot and dry summers and following springs with fewer growing degree days. Details on how we estimated each abiotic variable and the extent that each variable fluctuated over time can be found in Supporting Information S1.

The two biotic factors most likely to influence nutritional restriction in this system are changes in moose density (an index of intraspecific competition) and predation risk. For example, high levels of browsing associated with high herbivore density may reduce both the abundance and quality of forage by eliciting chemical defenses such as the production of certain PSMs (Nosko et al., 2020). Anti-predator behaviors may reduce time allocated to foraging and restrict access to high quality food and thereby influence diet quality (Barnier et al., 2014). Therefore, we included annual estimates of moose abundance (*moose*) and predation rate (*predation*) as candidate predictors. We also included a predictor variable where annual estimates of moose abundance were log-transformed *log(moose)* because models involving abundances often perform better when the variables are log-transformed. Predation rate is the proportion of prey population killed by predators and is indicative of temporal fluctuations in predation risk at the population level (Vucetich et al., 2011). We did not include wolf abundance as a predictor because wolf abundance and predation rate are highly correlated ($r = 0.81, df = 27, p < 0.001$), and moose population growth rates are more closely associated with predation rate than wolf abundance (Vucetich et al. 2011). For details on how we estimated moose abundance and predation rate see Supporting Information S1. The extent to which all seven predictor variables are correlated with one another is reported in Table S1.

We assessed which biotic and abiotic variables were important predictors of $UN{:}C_t$ and $GA{:}C_t$, using the *dredge* function of the *MuMIn* package in Program-R (Bartoń, 2018). The *dredge* function assessed models with all possible combinations of predictor variables included in a global model and ranked models on the basis of Akaike's Information Criterion corrected for small sample size (AICc). The global model contained all abiotic and biotic predictor variables (*snow, NAO, precip, GDD, temp, moose, log(moose*) and *predation*). Because we did not have *a priori* reason to think that any particular two-way interaction would be significant, we did not include any two-way interactions in the global model. Instead, we built *ad hoc* models evaluating all two-way interactions involving main effects that were included in the most parsimonious model identified by the *dredge* function.

We report the best model identified by the *dredge* function and all models with ΔAICc < 2. Moreover, because we present specific hypotheses about how each of the seven predictor variables may influence moose nutrition condition or investment in detoxification, we also report model coefficients and performance statistics for all univariate models. For additional context, we also report the null (intercept only) model. After identifying the most parsimonious models for predicting UN:C and GA:C, we visually checked plots of model residuals to assess assumptions of homoscedasticity, whether the residuals were normally distributed or autocorrelated, and whether any data points had a high leverage. We also formally tested the assumption that errors were normally distributed using both the Shapiro-Wilk and Kolmogorov-Smirnov test statistics. We followed this model building procedure for data representing the entire population (population-wide analysis) and subsequently on data for each region of the population (region-specific analyses).

Second, we assessed whether diet composition (proportion of deciduous species in winter diet) and diet diversity (evenness) were useful predictors of $UN{:}C_t$ or $GA{:}C_t$. We performed this assessment separately because data on diet composition is only available for a subset of study period (2004-2017, n = 13). We treated diet composition and diet diversity as biotic factors because they are determined by both the availability of forage types in the environment and moose foraging behavior.

Third, we used linear models to assess the extent that $UN{:}C_t$ and $GA{:}C_t$ co-varied over the 19-year study period in each region. Because C appears in both variables ($UN{:}C_t$ and $GA{:}C_t$), we also assessed the correlation between UN and GA (without using C as a "correction factor") for individuals in both regions. For this individual-level analysis, C does not need to be used as a baseline to correct for differences in an individual's body size, level of hydration or dilution due to snow because the estimates of UN and GA being compared were both derived from the same urine sample.

Results

Abiotic & Biotic Predictors

For the population-wide analysis, the single-most important predictor of temporal variation in UN:C was snow depth (*snow*, Table 1). The top model identified by the *dredge* function included three climatic variables (*snow, temp* and *precip*) and explained 66% of the interannual variation in $UN{:}C_t$ (Table 1). The next best models had ΔAICc values that were >3.18 (note these models are not reported in Table 1). Model coefficients indicate that $UN{:}C_t$ was greatest during winters with deep snow, during winters following warmer summers, and to a lesser extent during winters following a wet growing season (Table 1). Plots of model residuals and formal tests indicate that assumptions about homoscedasticity and normally distributed errors were met (Shapiro-Wilk: p = 0.1, Kolmogorov-Smirnov: p = 0.96). Moreover, model residuals were not autocorrelated, and no datapoints had high leverage. Multicollinearity was not a concern as the variables *snow, temp* and *precip* were not correlated (Table S1).

The *ad hoc* model which also included the two-way interaction *precip:temp* performed worse than the model that only included the main effects of *snow, temp* and *precip* (ΔAICc = 3.1, Table 1). By contrast, the *ad hoc* models which also included the two-way interactions *snow:precip* and *snow:temp* performed similarly to the model that only included the main effects of *snow, temp* and *precip,* insomuch as the ΔAICc between these models was less than 2 units (Table 1). Model coefficients for those two interaction terms indicate that the positive slope of the relationship between $UN{:}C_t$ and snow depth tended to be greater following a warmer summer and following a wetter growing season (see Fig. 1).

The results for the region-specific analyses were similar to the population-wide analysis (see Supporting Information S4). More precisely, the top model predicting $UN{:}C_t$ for moose in the eastern region included the variables *snow* and *temp,* but not *precip* (Table S3). The top model predicting $UN{:}C_t$ for moose in the western region included the variables *snow, temp, precip* and a two-way interaction between *snow:precip* (Table S4). In both regions, model coefficients suggest that $UN{:}C_t$ also tended to be higher during winters with deep snow and during winters following warmer summers.

Unlike nutritional restriction, investment in detoxification was not strongly associated with any of the variables examined. That inference is based on having observed that the null (intercept only) model was within 2 AICc units of the best models identified by the *dredge* function in both the population-wide analysis and region-specific analyses (Table 2, Table S5 and Table S6).

Winter diet, which is determined by both the availability of forage types in the environment and moose foraging behavior, was also not a useful biotic predictor of nutritional restriction or investment in detoxification (Supporting Information S5). More precisely, $UN{:}C_t$ was not correlated with either the proportion of diet comprised of deciduous forage or diet diversity (evenness) for moose in either region (Fig. S3a-b). There was also no strong evidence to suggest that $GA{:}C_t$ was correlated with the proportion of diet comprised of deciduous forage or diet diversity (evenness) in either region (Fig. S3c-d).

Spatio-Temporal Variation in UN:C and GA:C

Moose in the western region tended to have lower $UN{:}C_t$ and $GA{:}C_t$, indicating less nutritional restriction and less investment in detoxification, as compared to moose in the eastern region (Fig. 2). $UN{:}C_t$ was highly correlated between the eastern and western regions ($r = 0.67$, $p < 8.1\text{x}10^{-5}$, $df = 27$); whereas $GA{:}C_t$ was not correlated between the two regions ($r = 0.14$, $p = 0.57$, $df = 17$, see also Fig. 2).

Nutritional restriction appeared to increase as moose invested more in detoxification, given that $UN{:}C_t$ was positively correlated with $GA{:}C_t$ in both the population-wide analysis ($r = 0.65$, $p = 0.003$, $df = 17$, Fig. 3) and region-specific analysis (Fig. S4). For the population-wide analysis, $GA{:}C_t$ explained 42% of the interannual variance in $UN{:}C_t$ (Fig. 3) which suggests the energetic cost of detoxification is one mechanism that may cause nutritional condition to fluctuate at the population level.

These results are unlikely to be an artifact of correcting the concentrations of UN and GA in snow-urine samples with C for the following reasons. First, an analysis without correcting for C also indicated that UN and GA were significantly lower for moose in the western than the eastern region (UN: $F = 23.52$, $p < 1.5\text{x}10^{-6}$; GA: $F = 27.39$, $p < 2.2\text{x}10^{-7}$, see Fig. S5). Second, the relationship between UN and GA (without correcting for C) is also strongly positive when examined at the individual-level for moose living in both regions (Fig. S6). Third, there was no significant difference in concentrations of C between the two regions ($F = 0.17$, $p = 0.68$, see Supporting Information S6, Fig. S5a)

Discussion

Abiotic Predictors

Temporal variation in nutritional restriction (as indicated by UN:C) was importantly associated with abiotic factors (Fig. 1), but not biotic factors (moose density, predation risk or diet composition, Table 1). More precisely, nutritional restriction was greater for moose during winters with deeper snow, especially when winters with deep snow followed warmer summers or wetter growing seasons (Fig. 1). An adverse effect of deep snow on nutritional condition is probably due to the increased energetic cost of moving and the tendency for ungulates to restrict movement in deep snow (Moen, 1976; Parker et al., 1984), which may limit forage intake rates and the quality of ingested forage. Observing that nutritional condition was lower during winters with deep snow is also consistent with earlier research indicating that moose body mass (another index of condition) tended to be lower during winters with more snow (Hjeljord and Histøl, 1999).

Warmer summers may adversely affect the nutritional condition of moose (Fig. 1a) via some combination of mechanisms. First, warmer summer temperatures are thought to benefit an important parasite for moose, winter ticks (*Dermacentor albipictus*), which feed on the blood of their ungulate hosts over winter. Specifically, warmer temperatures during summer promote faster development of tick eggs and increased egg survival (Drew and Samuel 1987), and blood consumption by ticks is known cause protein deficits and substantial energetic costs for moose (Glines and Samuel 1989; Musante *et al.* 2007; Wünschmann *et al.* 2015). Second, warmer summers may have a negative effect on balsam fir, the primary winter forage species for moose. For example, warmer temperatures can reduce net photosynthesis and growth in balsam fir by up to 25% (Reich et al., 2015) and the abundance of balsam fir is predicted to decline in some regions as the climate continues to warm (Handler, 2014). Any such declines in balsam fir growth and abundance may represent reductions in the availability or quality of this important winter forage species for moose. (Our results (Fig. 2a) also suggests that the abundance or quality of forage available to moose may also be lower during wetter summers.) Third, higher temperatures can cause heat stress in moose (e.g., increased metabolic, heart and respiration rates), causing them to reduce food intake (Shively et al., 2019; Thompson et al., 2020) and seek thermal shelter in areas, such as dense conifer stands, where the abundance and quality of forage may be lower (van Beest and Milner, 2013). Additionally, body condition during summer is thought to be an important determinate of body condition throughout the rest of the year for large ungulates living in temperate climates (Cook et al., 2013). Consequently, the greater thermal stress, higher metabolic costs and reduced food intake experienced by moose during hot summers could result in them entering winter in a poorer nutritional state. Irrespective of what mechanism underlies the observed relationships, our results suggest that the adverse effect of warmer summers (or wetter growing seasons) on nutritional condition tends to be exacerbated during winters with deep snow (Fig. 1).

Investment in detoxification (as indicated by GA:C) was not associated with any of the abiotic factors examined here. That absence of a relationship may be explained by one or both of the following considerations. Although abiotic factors such as temperature and precipitation can affect concentrations of PSMs in some forage species, they may have only a negligible influence on the PSM profiles of balsam fir (Berini et al., 2018), the primary forage species for moose. It is also possible that moose may alter their foraging behaviour (i.e., altering intake rates) to compensate for the negative effects of abiotic factors on PSM concentrations in certain forage species.

These results offer insights relevant for understanding how climate change may influence herbivore populations over the next few decades. For example, observing that nutritional restriction was greater during winters with deeper snow (Fig. 1) suggests that the influence of climate warming on snow conditions may have an important influence on ungulate populations. Specifically, as the climate continues to warm it could potentially benefit moose populations by causing earlier snowmelt in spring. However, climate change is also expected to increase the likelihood of severe storms and heavy precipitation events in winter (Hayhoe et al., 2010), which may negatively affect moose populations in regions where air temperatures remain cold enough for precipitation to fall as snow. For example, in the Great Lakes region, there is expected to be an increase in lake-effect snowfall during winter, at least in the near future (Burnett et al. 2003). It is also possible that the effect of climate warming on the formation of hard crusts on the snow surface (due to freeze-thaw cycles) may affect moose habitat selection and ultimately their nutritional condition. For example, during annual winter surveys of moose abundance we observed that moose tend to concentrate in dense coniferous forest (where the quality of food may be lower) once crusts have formed on the snow surface (Peterson et al., 2018). Lastly, our results (Table 1 and Fig. 1) suggest that the extent to which moose are impacted by changing snow conditions will also depend on how climate change influences temperature and precipitation during the summer. However, it is relevant that across much of North America, climate warming has been least pronounced during summer (Vincent et al., 2015; Vose et al., 2017). Moreover, no significant changes in precipitation are expected during the summer (Hayhoe *et al.* 2010). For those reasons, changes to snow conditions during winter are likely to be the most important mechanism by which climate warming will influence moose populations in the United States.

Biotic Predictors

Neither nutritional restriction nor investment in detoxification were related to the biotic factors, density or predation rate (Table 1). The importance of this results lies in understanding the interconnected nature among density, predation and intraspecific competition for forage. First, moose density on Isle Royale regularly exceeds 2 moose/km^2, which is high compared to other moose populations in comparable habitat (Lavsund et al., 2003). Consequently, the lack of relationship with density is unlikely the result of predation suppressing moose density below the point at which intraspecific competition becomes important.

Second, although there is a tendency for competition to increase with density, there is also a tendency for predation rate to decrease with density (Supporting Information S7). The countervailing nature of those forces results in population growth being independent of density over a wide range of densities for this moose population (Supporting Information S7). Consequently, the absence of a relationship between density and indicators of nutritional condition cannot simply be interpreted as the absence of a relationship with intraspecific competition. The more appropriate inference is that density and predation together represent the most important biotic influences on this population, and neither is a good predictor of nutritional condition for moose. It remains to be seen whether the absence of those relationships is characteristic of systems where predation is not a strong force.

Third, it is possible that high herbivore densities tend to have a stronger effect on the quantity, rather than quality, of food available to moose. In support of that idea, experiments suggest that browsing had no effect on concentrations of condensed tannins in balsam fir saplings; and there is conflicting evidence about whether browsing influenced phenol concentrations (Nosko et al., 2020; Warbrick et al., 2020). Therefore, if nutritional condition and investment in detoxification are primarily influenced by the nutritional quality of food consumed, then it may explain why moose density was not a useful predictor of UN:C or GA:C.

Finally, the lack of relationship between predation rate and indices of nutritional condition is relevant for understanding the non-lethal and lethal effects of predation. An important aspect of the non-lethal effects of predation involves prey altering their foraging behaviour as a means to reduce predation risk, and such anti-predator responses can involve significant physiological or energetic costs for prey (Creel and Christianson, 2008). However, the lack of relationship between predation rate and indices of nutritional condition suggests that any risk-sensitive foraging behaviour that occurred during years of higher predation risk did not significantly impair the average nutritional condition of moose during winter. By contrast, previous research suggests that predation has a strong influence on moose population growth rates (Vucetich et al., 2011; Peterson et al., 2014). Together, these results suggest that non-lethal effects of predation are probably less important than lethal effects (direct mortality) in driving fluctuations in the abundance of moose on Isle Royale.

Nutrition & Diet Composition

Contrary to our hypotheses, neither nutritional restriction nor investment in detoxification were related to the proportion of the diet comprised of deciduous forage or the diversity of forage species consumed, at least not at the population level. Not finding strong relationships with the composition of different forage species in the diet is probably because forage quality (i.e., concentrations of crude protein and PSMs) can vary substantially among plants of the same species and among parts of the same plant (Terra-Berns, 1993; Frye et al., 2013; Ulappa et al., 2014). For example, PSM profiles can vary among plants of the same species due to differences in tree height, the age of foliage, light availability, and soil pH (Nosko et al., 2020; Warbrick et al., 2020). Therefore, even if herbivores consume a similar quantity of a given forage species, the amount of PSMs or protein that they consume may differ depending on which particular trees or parts of the trees they browsed. Studies involving feeding trials on captive animals suggest that herbivores tended to select plants with a higher crude protein content or lower PSM concentration (Guglielmo et al., 1996; Sorensen et al., 2005a; Somers et al., 2008). Therefore, if free-ranging herbivores also exhibit such fine-scale selective foraging behavior, then it could weaken the observed relationship between diet composition, nutritional condition, and investment in detoxification. The broader significance of these results is that they suggest interannual variation in nutritional condition for free-ranging herbivores may be more closely related to fine-scale variation in forage quality (concentrations of protein and PSMs) than diet composition.

Nutrition Restriction & Detoxification of PSMs

Although our analysis did not identify any significant predictors of temporal variation in GA:C, we found evidence of a strong positive relationship between UN:C and GA:C in both the eastern and western regions (Fig. 3). This finding is consistent with detoxification of PSMs being energetically costly (Sorensen et al., 2005b; Au et al., 2013), such that it impairs nutritional condition (Villalba et al., 2002). Prior work has shown that *individuals* investing more in detoxification tend to be in lower nutritional condition (68 individuals sampled over 2 years, Parikh et al. 2017). However, our work goes considerably further by showing that GA:C and UN:C covary at the *population*-level and over long periods of time (nearly two decades) that are salient to population dynamics (Fig. 3). Indeed, mean GA:C explained over 40% of the interannual variation in UN:C over the 19-year study period. Given that previous research suggests nutritional condition is an important determinant of reproductive success and survival (Parker et al., 2009), our results highlight the potential for temporal variation in PSMs intake and detoxification costs to have an important influence on herbivore population dynamics.

Regional Differences

The average level of nutritional restriction and investment in detoxifying PSMs varied significantly over relatively small spatial scales (i.e., between two regions separated by less than 50km) being lower for moose in the western region (Fig. 2). Because UN:C and GA:C were not closely related to indices of diet composition it suggests that the average nutritional quality of primary forage species, may be greater in the western region. The extent that concentrations of PSM vary spatially and temporally in this system is not known, but one potentially important difference is that the protein content of balsam fir tends to be greater in the western region (Fig. S2). Such differences in protein content are likely to be important because balsam fir and some other forage species contain condensed tannins (Sauvé and Côté, 2007; Nosko et al., 2020; Warbrick et al., 2020) that bind with protein (Barbehenn and Constabel, 2011). Additionally, when herbivores consume high concentrations of PSMs whilst on low-protein diets it can cause a negative nitrogen balance (Au et al. 2013). Therefore, it is plausible that having access to forage with a higher crude protein content reduces the energetic (i.e., loss of glucose via GA) and protein costs of detoxification. This is pertinent because spatial variation in the PSMs and protein content of forage at scales relevant to the population dynamics of vertebrate herbivores is likely common and such spatial variation in may contribute importantly to spatial variation in reproductive success of vertebrate herbivores (DeGabriel et al., 2009; McArt et al., 2009).

In summary, this study offers several insights relevant for understanding herbivore population dynamics. First, our results (Fig. 1) are a strong indication that the influence of nutritional condition on population dynamics is importantly mediated by climatic factors. Second, although our results suggest that predation risk may not elicit risk-sensitive behaviours in moose that affect their nutritional condition, temporal variation in nutritional condition and predation may still interact (albeit with some time-lag) to influence on herbivore population dynamics. That inference is based on observing that individuals in substandard condition tend to be more vulnerable to predation (Temple, 1987; Mech and Boitani, 2003; Genovart et al., 2010), and that predation has an important effect on herbivore population growth rates ((Vucetich et al., 2011; Peterson et al., 2014), see also Fig. S7). Third, the strong relationship that we observed between UN:C and GA:C (Fig. 3, Fig. S4 and Fig. S6) suggests that the influence of nutritional condition on population dynamics is likely to be importantly mediated by interannual variation in PSMs intake and detoxification costs (DeAngelis et al., 2015). Lastly, although diet composition may be easier to measure than diet quality, our results suggest that for free-ranging herbivores, diet quality may be of greater consequence for population dynamics than the species composition of diet.

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Tables

Table 1. Performance of linear models predicting the mean annual ratio of urea nitrogen to creatinine ($UN{:}C_t$, an indicator of nutritional restriction) in samples of urine deposited in the snow by moose (*Alces alces*) in Isle Royale National Park collected over a 29-year period. This analysis is referred to as the population-wide analysis as it does not distinguish between moose living in the eastern and western regions of the study site. The candidate predictors were: mean snow depth (*snow*); North Atlantic Oscillation, an indicator of winter severity (*NAO*); mean summer temperature (*temp*); total precipitation in May – August (*precip*); cumulative number of growing degree days reached by mid-June (*GDD*); moose abundance (*density*); and predation risk (*predation*). R^2 represents the total amount of variation in $UN{:}C_t$ explained by the model and R^2-adj is a modified version of R^2 that has been adjusted for the number of predictors in the model. ΔAICc is the differences in AICc between the model of interest and the model with the lowest AICc and *w* is the Akaike weight which indicated the relative likelihood of the model. The model in bold font was the top model identified by the ***dredge*** function in Program R. We also built three *ad hoc* models to assess whether there were any significant two-way interactions between the main effects included in the top model.

Predictor variable(s)	Model coefficient (standard error)	R^2	R^2-adj	ΔAICc	*w*
null	-	-	-	23.97	<0.01
snow	0.04 (0.01)	0.24	0.21	18.65	<0.01
NAO	-0.01 (0.04)	<0.01	<0.01	26.34	<0.01
precip	0.06 (0.03)	0.09	0.06	23.58	<0.01
GDD	<0.01 (<0.01)	<0.01	<0.01	26.46	<0.01
temp	0.14 (0.06)	0.18	0.15	20.83	<0.01
density	0.02 (0.2)	<0.01	<0.01	26.46	<0.01
predation	-0.91 (1.48)	0.01	<0.01	26.07	<0.01
snow + temp	0.05 (0.01), 0.19 (0.05)	0.53	0.50	7.18	0.01
snow + temp + precip	**0.05 (0.01), 0.20 (0.04), 0.07 (0.02)**	**0.66**	**0.62**	**1.15**	**0.22**
snow + temp + precip + temp:precip	0.05 (0.01), 0.11 (0.31), -0.28 (1.22), 0.01 (0.02)	0.66	0.60	4.26	0.05
snow + temp + precip + snow:precip	-0.08 (0.07), 0.19 (0.04), -0.07 (0.07), 0.01 (<0.01)	0.70	0.65	0.25	0.34
snow + temp + precip + snow:temp	-0.99 (0.53), -0.07 (0.14), 0.06 (0.02), 0.02 (0.01)	0.70	0.66	0	0.39

Table 2. Performance of linear models predicting the mean annual ratio of glucuronic acid to creatinine ($GA{:}C_t$, an indicator of investment in detoxification of plant secondary metabolites) in samples of urine deposited in the snow by moose (*Alces alces*) in Isle Royale National Park over a 19-year period. This analysis is referred to as the population-wide analysis in the main text as it does not distinguish between moose living in the eastern and western regions of the study site. The model in bold font was the top model identified by the ***dredge*** function in Program R and * indicates models that were within 2 AICc units of the top model. All other details are identical to those described in Table 1. The only exception is that we did not build *ad hoc* models to assess whether there were any significant two-way interactions between the main effects because the top model identified by the dredge function performed equivalently to the null model.

Predictor variable(s)	Model coefficient (standard error)	R^2	R^2-adj	dAICc	w
null *	-	-	-	0.77	0.14
snow	0.13 (0.22)	0.02	<0.01	3.23	0.04
NAO	-0.25 (0.65)	0.01	<0.01	3.45	0.04
precip	0.26 (0.53)	0.01	<0.01	3.34	0.04
GDD	<0.01 (0.01)	<0.01	<0.01	3.61	0.03
temp *	1.87 (1.04)	0.16	0.11	0.29	0.18
density	0.72 (3.29)	<0.01	<0.01	3.56	0.03
predation	-20.17 (19)	0.06	0.01	2.40	0.06
snow + temp*	**0.40 (0.22), 2.83 (1.11)**	**0.30**	**0.22**	**0**	**0.20**
temp + predation *	1.90 (1.03), -21.05 (17.78)	0.23	0.13	1.95	0.08
snow + temp + predation *	0.40 (0.22), 2.87 (1.09), -21.35 (16.55)	0.37	0.25	1.76	0.08
snow + temp + precip *	0.45 (0.22), 3.1 (1.11), 0.57 (0.47)	0.37	0.24	1.98	0.08

Figures

Fig. 1. Mean annual values of the ratio of urea nitrogen to creatinine (UN:C, nutritional restriction) for samples of urine-soaked snow collected from moose (*Alces alces*) in two regions of Isle Royale National Park shown in relation to mean snow depth. In panel (a) lines depict predictions from a regression model that includes the main effects *snow*, *temp*, *precip* and an interaction between *snow:temp* (see Table 1). More precisely, lines depict predictions across the observed range of snow depth values, where *precip* was fixed at the median value and *temp* was fixed at the 15th percentile (dashed line) and at the 85th percentile (solid line). In panel (b) lines depict predictions from a regression model that includes the main effects *snow*, *temp*, *precip* and an interaction between *snow:precip* (see Table 1). More precisely, lines depict predictions across the observed range of snow depth values, where *temp* was fixed at the median value and *precip* was fixed at the 15th percentile (dashed line) and at the 85th percentile (solid line).

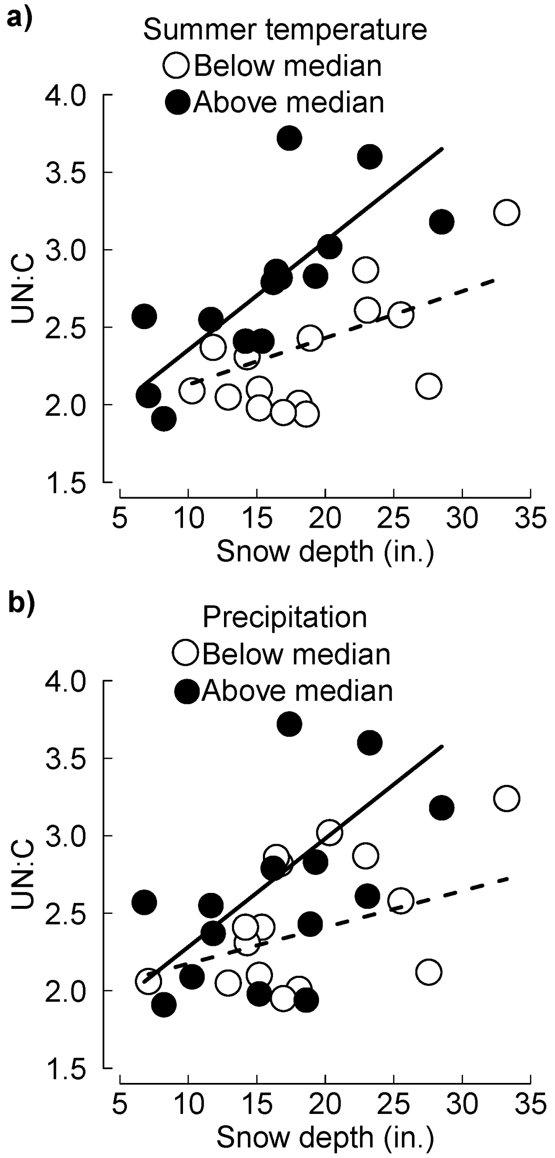


Fig. 2. Spatial variation in the mean annual ratio of urea nitrogen to creatinine (UN:C, nutritional restriction) over a 29-year period and the mean annual ratio of glucuronic acid to creatinine (GA:C, energetic investment in detoxification) over a 19-year period for samples of urine-soaked snow collected from moose (*Alces alces*) in two regions of Isle Royale National Park. Observations above the 1:1 reference (dotted) line indicate years where mean annual UN:C and GA:C were greater in the eastern region. The solid line in panel (a) is a linear regression. Year-specific sample sizes for UN:C and GA:C are given Supporting Information S3.

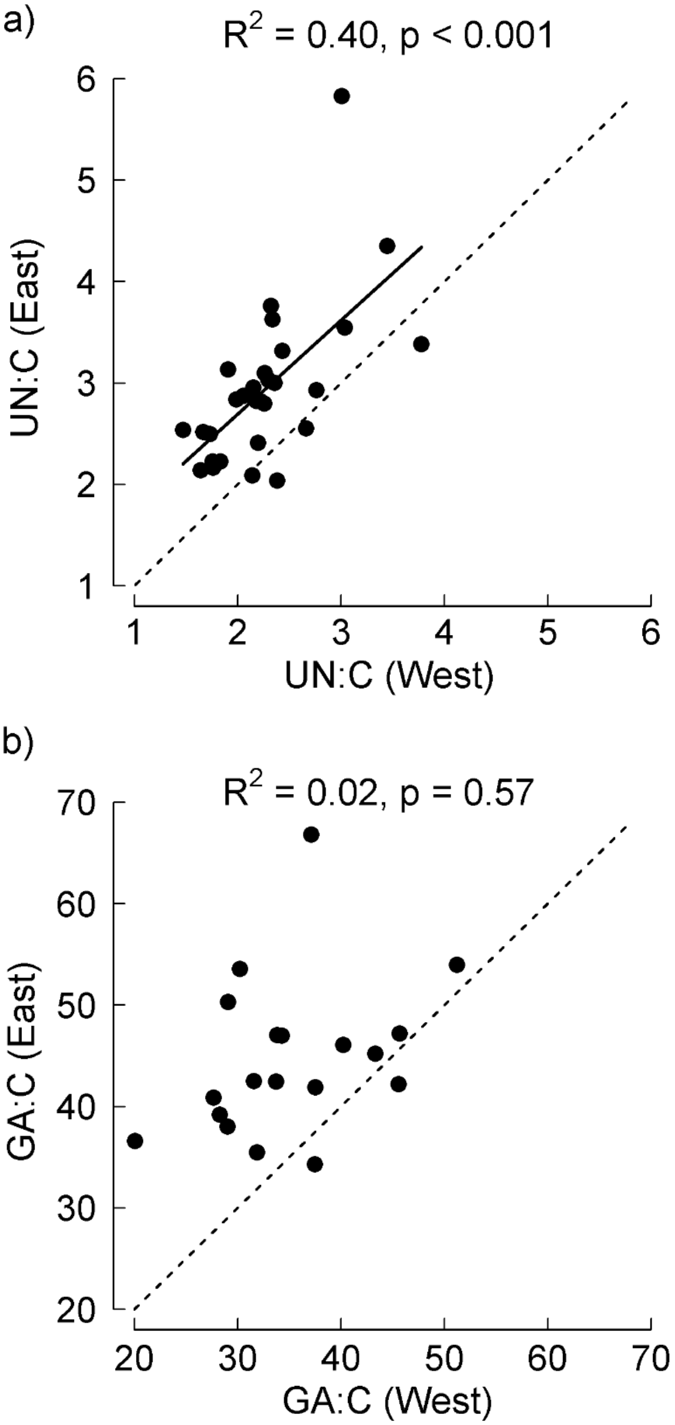
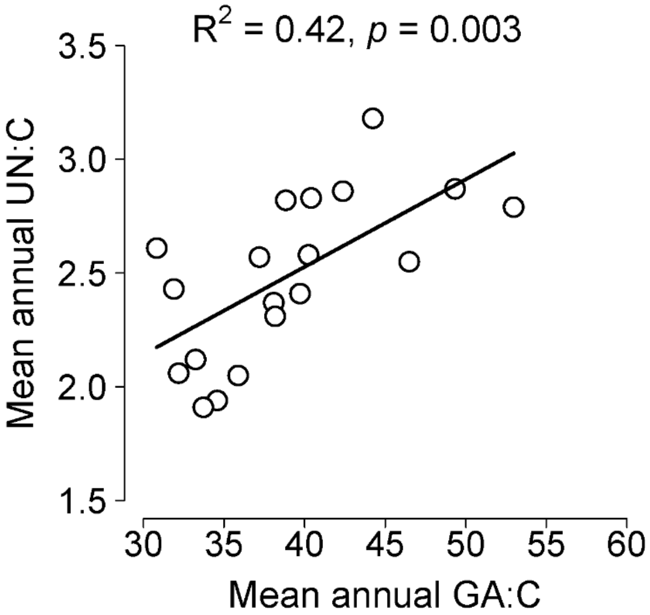


Fig. 3. Relationship between the ratio of urea nitrogen to creatinine (UN:C, an indicator of nutritional restriction) and the ratio of glucuronic acid to creatinine (GA:C, an indicator of investment in detoxification) for samples of urine-soaked snow collected from moose (*Alces alces*) in Isle Royale National Park. Points depict mean annual values for the entire population (east and west regions combined) and the line represents the best fit regression (with an estimated slope of 0.04 ± SE 0.01) over a 19-year period (1994, 1997, 2000-2015, 2017).



Supporting Information

The nutritional condition of moose co-varies with climate, but not with density, predation risk, or diet composition

S1 – Abiotic and Biotic Predictors of Temporal Variation in UN:C and GA:C

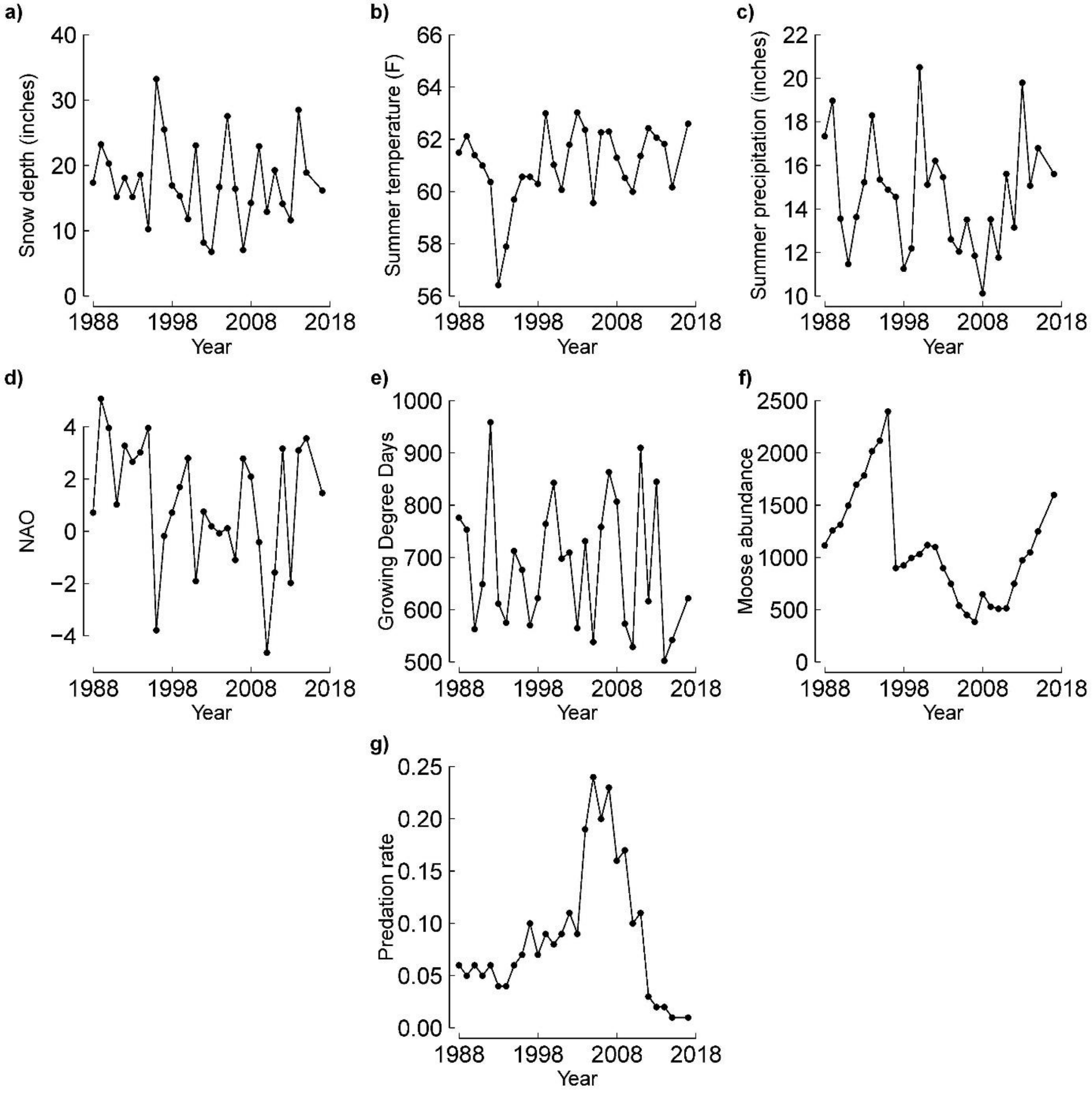


Fig. S1 Temporal variation in seven abiotic and biotic variables evaluated as candidate predictors of interannual variation in UN:C (an indicator of nutritional restriction) and GA:C (an indicator of investment in detoxification) in Isle Royale National Park over a 29-year period. A description of each variable and how it was estimated is provided below.

Snow – We measured snow depth (inches) on a daily basis between mid-January to early March each year. We then averaged daily measurements to generate an estimate of mean snow depth for each year.

NAO – We obtained station-based measurements of the North Atlantic Oscillation (NAO) index for each winter (January-March) from the National Center for Atmospheric Research (Hurrell, 1995).

Precip - We obtained estimates of the total precipitation (inches) during the growing season (May – Aug) from a local weather station in north-eastern Minnesota, located approximately 40-60km from Isle Royale (Western Regional Climate Center, 2016).

GDD – Growing degree days (GDD) indicates plant phenology and the timing of spring "green-up" (Daughtry, Cochran, & Hollinger, 1984; Herfindal, Saether, Solberg, Andersen, & Høgda, 2006). For each day of the year, we calculated the number of growing degree days by subtracting a base temperature (40°F) from the mean of the daily maximum and minimum temperatures (°F). We then estimated the cumulative number of growing degree days reached by June 16th for each year. Daily maximum and minimum temperatures were obtained from local weather stations located 40-60km from Isle Royale (NCEI, 2019). Maximum temperatures exceeding 86°F were recorded as 86°F and minimum temperatures less than 40°F were recorded as 40°F (McMaster & Wilhelm, 1997).

Temp – We obtained estimates of the mean daily temperature during the hottest months (July and September) from a local weather station in north-eastern Minnesota, located approximately 40-60km from Isle Royale (Western Regional Climate Center, 2016).

Moose - Moose abundance was estimated annually from aerial surveys conducted between late January and February each year throughout the study period (Gasaway, Dubois, Reed, & Harbo, 1986; Peterson & Page, 1993).

Predation - We used predation rate as an annual index of predation risk. Predation rate was estimated as *Predation* = *KR* x *P*/*N*, where *KR* (kill rate) is an estimate of the number of moose killed per predator, per time unit, *N* represents moose abundance and *P* represents wolf abundance using the methods described in (Vucetich, Hebblewhite, Smith, & Peterson, 2011). Wolf abundance was estimated annually by aerial census from a fixed-wing aircraft (Peterson & Page, 1988).

Table S1. Matrix of correlations between all predictor variables used in the analysis in the main text. Values in the upper left half of the table are correlation coefficients (*r*) and values in lower left half of the table are the associated p-values. Correlations that are statistically significant are indicated with bold font and an asterisk.

Predictor	*snow*	*NAO*	*precip*	*GDD*	*temp*	*moose*	*predation*
snow	-	-0.16	-0.02	-0.31	-0.22	0.20	-0.04
NAO	0.40	-	0.16	0.03	-0.03	0.25	-0.28
precip	0.93	0.40	-	0.15	-0.06	0.34	**-0.49***
GDD	-0.31	0.87	0.43	-	0.28	-0.08	0.18
temp	0.24	0.87	0.75	0.14	-	**-0.43***	0.13
moose	0.30	0.19	0.07	0.70	**0.02***	-	**-0.62***
predation	0.85	0.14	**0.01***	0.36	0.50	**<0.001***	-

S2 – Regional Variation in Forage Quality

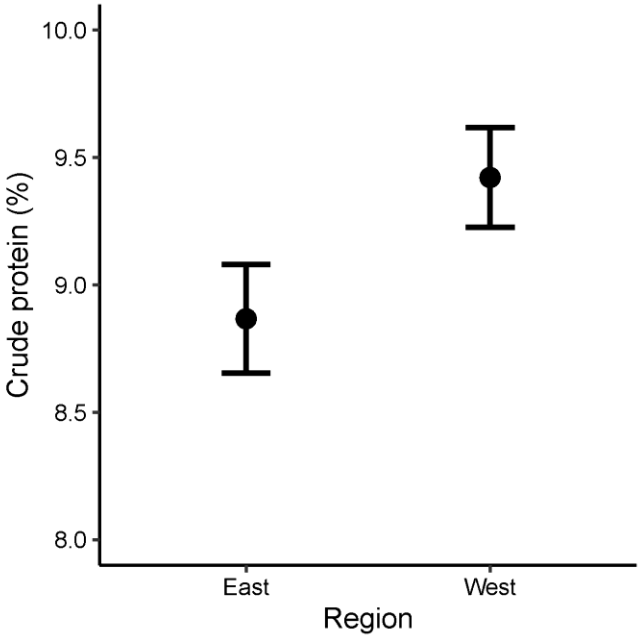


Fig. S2. Regional variation in the crude protein content (% dry weight) of balsam fir saplings sampled in winter (mid-January to late-February) over a 3-year period (2013-2015) in two regions (east and west) of Isle Royale National Park. Analysis of variance (ANOVA) suggests that the concentration of crude protein in balsam fir saplings is significantly higher in the western region (0.55 ± 0.15, *f* = 13.35 *df* = 1, 200; *p* = 0.001).

S3 - Collection of Urine & Fecal Samples from Moose.

Table S2. The number of moose-urine samples used to derive estimates of mean annual UN:C and GA:C for each of the two regions (east and west) in Isle Royale National Park. The smaller sample sizes for GA:C (compared to UN:C) is because GA in urine wasn't recognized as being a useful indicator of detoxification of PSMs until relatively recently (Marsh, Wallis, Andrew, & Foley, 2006) and we do not have archived snow-urine samples that would allow us to observed GA:C for years aside from those reported on here.

Year	UN:C		GA:C	
	East	West	East	West
1988	50	16	-	-
1989	82	37	-	-
1990	51	27	-	-
1991	59	30	-	-
1992	47	24	-	-
1993	58	28	-	-
1994	49	20	20	19
1995	117	54	-	-
1996	143	56	-	-
1997	47	25	20	18
1998	30	14	-	-
1999	73	36	-	-
2000	53	20	20	20
2001	101	40	20	16
2002	74	40	21	21
2003	41	24	23	17
2004	89	31	17	20
2005	50	20	15	25
2006	61	35	19	18
2007	40	12	12	22
2008	65	13	12	25
2009	66	30	17	20
2010	84	58	19	19
2011	94	66	20	20
2012	104	74	18	22
2013	112	67	22	19
2014	149	100	31	8
2015	74	20	20	20
2017	60	38	38	22

Each year, we collected snow-urine and pellet samples from multiple locations in both regions of the island (see Table S1). Each pellet & snow-urine sample was placed in its own sealed plastic bag and stored at -20C until it could be analysed. Most snow-urine samples were collected within 72 hours of deposition (date of deposition was determined on the basis of snow conditions). It is plausible that nutritional restriction might increase as the winter progresses such that variation in the date that samples were collected each year could potentially bias the results of analyses. However, that concern is alleviated by linear regression models suggesting that the Julian day of sample collection explained less than 2% of the variation in concentrations of UN, C, and GA in snow-urine samples. Moreover, the sampling period did not vary among years. Between 2004-2011, fecal pellets were collected from fixed locations in both the eastern and western regions as part of another study (Hoy et al. 2019). After 2012, we collected fecal pellets in the same way that we collected snow-urine samples (i.e., by following the tracks of the individual moose).

S4 – Regional Analysis of Nutritonal Ecology

Table. S3 Performance of linear models predicting the mean annual ratio of urea nitrogen to creatinine (UN:C) for moose in the EASTERN region of the study site. The model in bold font was the top model identified by the *dredge* function in Program-R and * indicates models that were within 2 AICc units of the top model. All other details are the same as those presented in Table 1 and Table 2 of the main text. Although the best performing model identified by the dredge function met assumptions of homoscedasticity and had no data points with high leverage. There was conflicting evidence about whether errors were normally distributed (Shapiro-Wilk: $p < 0.001$, Kolmogorov-Smirnov: $p = 0.14$).

Predictor variable(s)	Model coefficient (standard error)	R^2	R^2-adj	ΔAICc	*w*
null	-	-	-	5.99	0.01
snow	0.03 (0.02)	0.11	0.08	5.11	0.02
NAO	-0.01 (0.05)	<0.01	<0.01	8.43	<0.01
precip	0.06 (0.05)	0.05	0.01	7.09	0.01
GDD	<0.01 (<0.01)	0.02	<0.01	7.90	<0.01
temp	0.17 (0.08)	0.14	0.11	4.16	0.02
moose	0.09 (0.26)	<0.01	<0.01	8.37	<0.01
predation	-0.68 (2.01)	<0.01	<0.01	8.37	<0.01
snow + temp*	**0.05 (0.02), 0.21 (0.08)**	**0.32**	**0.27**	**0**	**0.20**
*snow + temp + precip**	0.05 (0.02), 0.22 (0.07), 0.06 (0.04)	0.38	0.31	0.06	0.19
*snow + temp + GDD**	0.05 (0.02), 0.2 (0.08), <0.01 (<0.01)	0.35	0.27	1.82	0.08
*snow + temp + moose**	0.04 (0.02), 0.24 (0.08), 0.25 (0.24)	0.35	0.27	1.68	0.09
snow + temp + snow:temp	-1.50 (1.00), -0.19 (0.27), 0.03 (0.02)	0.38	0.30	0.33	0.17
snow + temp + precip + temp:precip	0.05 (0.02), 0.41 (0.55), 0.8 (2.19), -0.01 (0.04)	0.39	0.29	3.14	0.04
snow + temp + precip + snow:precip	-0.05 (0.13), 0.22 (0.07), -0.03 (0.14), 0.01 (0.01)	0.40	0.30	2.66	0.05
snow + temp + precip + snow:temp	-1.36 (0.98), -0.15 (0.27), 0.06 (0.04), 0.02 (0.02)	0.43	0.34	0.89	0.13

Table. S4 Performance of linear models predicting the mean annual ratio of urea nitrogen to creatinine (UN:C) for moose in the WESTERN region of the study site. All other details are the same as those presented in Table 1 and Table 2 of the main text. Plots of model residuals and formal statistical tests associated with the best performing models indicate that statistical assumptions about homoscedasticity and normality were met (Shapiro-Wilk: $p > 0.63$, Kolmogorov-Smirnov: $p > 0.77$) and no datapoints were found to have high leverage.

Predictor variable(s)	Model coefficient (standard error)	R2	R2-adj	ΔAICc	*w*
null	-	-	-	21.21	<0.01
snow	0.04 (0.01)	0.24	0.21	15.85	<0.01
NAO	-0.02 (0.04)	0.01	<0.01	23.53	<0.01
precip	0.06 (0.03)	0.10	0.07	20.68	<0.01
GDD	<0.01 (<0.01)	<0.01	<0.01	23.69	<0.01
temp	0.11 (0.06)	0.11	0.08	20.38	<0.01
moose	-0.07 (0.19)	<0.01	<0.01	23.58	<0.01
predation	-0.16 (1.44)	<0.01	<0.01	23.70	<0.01
snow + temp	0.04 (0.01), 0.15 (0.05)	0.44	0.40	9.61	0.01
snow + temp + precip*	**0.05 (0.01), 0.16 (0.04), 0.06 (0.02)**	**0.57**	**0.51**	**5.20**	**0.06**
*snow + temp + precip + moose**	0.05 (0.01), 0.14 (0.05), 0.08 (0.03), -0.19 (0.15)	0.59	0.52	6.52	0.03
snow + temp + precip + temp:precip	0.04 (0.01), -0.04 (0.33), -0.71 (1.31), 0.01 (0.02)	0.57	0.50	8.00	0.02
snow + temp + precip + snow:precip	-0.15 (0.07), 0.15 (0.04), -0.14 (0.07), 0.01 (0)	0.67	0.62	0	0.86
snow + temp + precip + snow:temp	-0.51 (0.61), 0.01 (0.16), 0.06 (0.02), 0.01 (0.01)	0.58	0.51	7.43	0.02

Table. S5 Performance of linear models predicting the mean annual ratio of glucuronic acid to creatinine (GA:C, an indicator of investment in detoxifying PSM) for moose in the EASTERN region of the study site. All other details are the same as those presented in Table 1 of the main text.

Predictor variable(s)	Model coefficient (standard error)	R^2	R^2-adj	ΔAICc	*w*
null*	**-**	**-**	**-**	**0**	**0.31**
snow	0.19 (0.33)	0.02	<0.01	2.50	0.09
NAO	-0.56 (0.95)	0.02	<0.01	2.40	0.09
precip	-0.17 (0.78)	<0.01	<0.01	2.80	0.08
GDD	0.01 (0.02)	0.02	<0.01	2.40	0.09
*temp **	1.9 (1.59)	0.08	0.02	1.30	0.16
moose	-1.79 (4.8)	0.01	<0.01	2.70	0.08
Predation	12.51 (28.57)	0.01	<0.01	2.60	0.09

Table S6 Performance of linear models predicting the mean annual ratio of glucuronic acid to creatinine (GA:C, an indicator of investment in detoxifying PSM) for moose in the WESTERN region of the study site. All other details are the same as those presented in Table 1 and Table 2 of the main text.

Predictor variable(s)	Model coefficient (standard error)	R^2	R^2-adj	ΔAICc	*w*
null *	-	-	-	1.30	0.12
snow	0.04 (0.27)	<0.01	<0.01	4.10	0.03
NAO	0.19 (0.78)	<0.01	<0.01	4.10	0.03
precip	0.4 (0.63)	0.02	<0.01	3.70	0.04
GDD	-0.01 (0.01)	0.01	<0.01	3.90	0.03
temp *	1.9 (1.26)	0.12	0.07	1.70	0.10
density	1.51 (3.89)	0.01	<0.01	4.00	0.03
predation *	-41.68 (20.97)	0.19	0.14	0.20	0.20
***predation + moose* ***	**-83.04 (30.5), -9.1 (5.12)**	**0.32**	**0.24**	**0**	**0.22**
*predation + temp**	-42.59 (19.87), 1.97 (1.15)	0.23	0.13	0.20	0.20

S5 - Diet Composition and Nutritonal Ecology

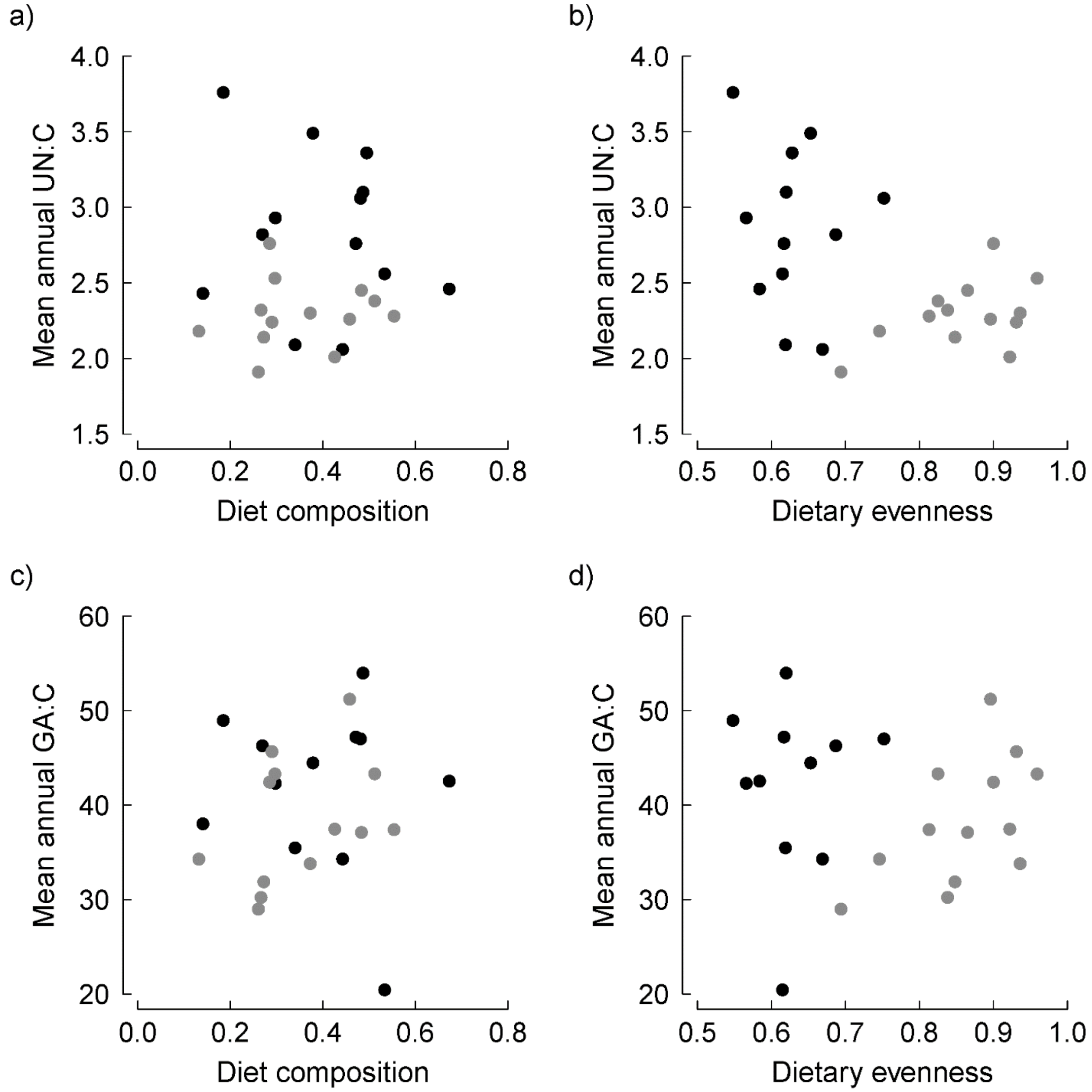


Fig. S3 Mean annual values of the ratio of urea nitrogen to creatinine ($UN:C_t$), an indicator for nutritional restriction (DelGiudice, 1995), and the ratio of glucuronic acid to creatinine ($GA:C_t$), an indicator for investment in detoxifying plant secondary metabolites (Guglielmo, Karasov, & Jakubas, 1996; Servello & Schneider, 2000) for moose in two regions of Isle Royale National Park. The eastern region is denoted in black and the western region in grey. $UN:C_t$ and $GA:C_t$ were assayed from samples of urine-soaked snow and are shown in relation to diet composition (proportion of the diet comprised of deciduous, as opposed to coniferous forage) and an indicator of diet diversity (dietary evenness). Linear models indicated that $UN:C_t$ was not associated with the proportion of the diet comprised of deciduous forage (East: p = 0.58, West: p = 0.71; df = 11; see panel a), nor with diet diversity in either region (East: p = 0.72, West: p = 0.10; df = 11; see panel b). Linear models also indicate that $GA:C_t$ was not associated with the proportion of the diet comprised of deciduous forage in either region (East: p = 0.98, West: p = 0.21; df = 11; see panel c), nor with diet diversity in eastern region (East: p = 0.62, West: p = 0.05; df = 11; see panel d).

S6 – Regional Variation in Urinary Metabolites

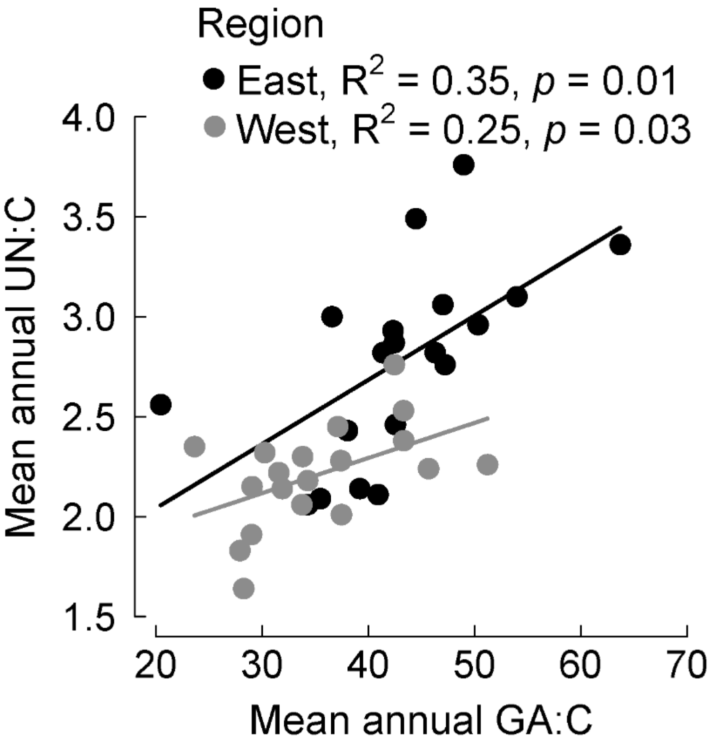


Fig. S4. Relationship between the ratio of urea nitrogen to creatinine (UN:C, an index of nutritional restriction) and the ratio of glucuronic acid to creatinine (GA:C, an index of investment in detoxification) for samples of urine-soaked snow collected from moose in two regions of Isle Royale National Park. Points depict mean annual values for moose in the eastern (black) and western (grey) region of the study site and the line represents the best fit regressions (with an estimated slope of 0.03 ± SE 0.01 in the eastern region and 0.02 ± 0.007 in the western region) over a 19-year period (1994, 1997, 2000-2015, 2017).

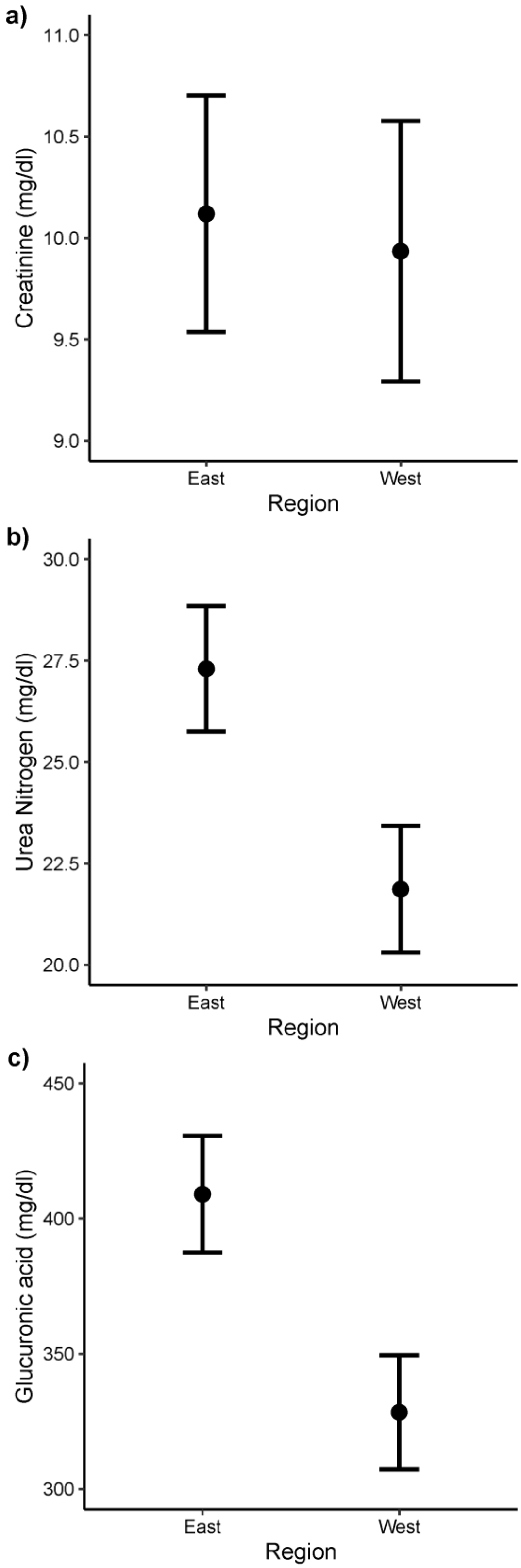

a)
Creatinine (mg/dl)
11.0
10.5
10.0
9.5
9.0
East
West
Region
b)
Urea Nitrogen (mg/dl)
30.0
27.5
25.0
22.5
20.0
East
West
Region
c)
Glucuronic acid (mg/dl)
450
400
350
300
East
West
Region

Fig. S5 Regional variation in the concentration of creatinine (a), urea nitrogen (b) and glucuronic acid (c) excreted in the urine of moose living in the eastern or westerns region of Isle Royale National Park. These urinary metabolites were assayed from samples of urine-soaked snow collected in winter (Jan-Feb) over a 29-year period. Whilst there was no significant difference in creatinine between the two regions (-0.18 ± 0.44, $p = 0.68$), both urea nitrogen and glucuronic acid were significantly lower for moose in the western region (UN: -5.43 ± 1.12, $p = 1.5\text{x}10^{-6}$, GA: -80.60 ± 15.4, $p = 2.16\text{x}10^{-7}$).

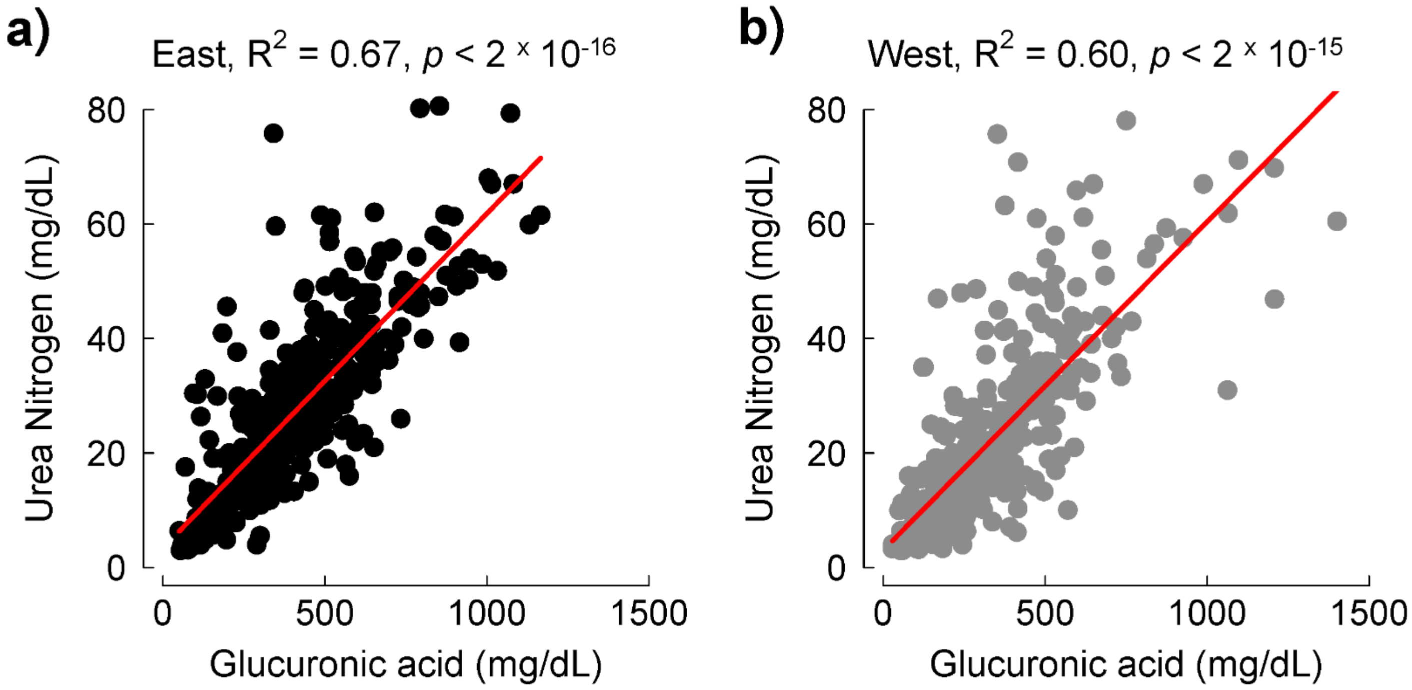


Fig. S6. Relationship between urea nitrogen and glucuronic acid in samples of urine-soaked snow collected from moose in two regions of Isle Royale National Park. Points depict values of individual moose in the eastern (Panel a, *n* = 384) and western (Panel b, *n* = 371) region of the study site. The line represents the best fit regressions (with an estimated slope of 0.03 ± SE 0.01 in the east and 0.02 ± 0.007 in the west) over a 19-year period (1994, 1997, 2000-2015, 2017).

S7 – Countervailing Effects of Predation and Density Dependence

Predation is a fundamentally density-dependent process insomuch as predation (by wolves) rapidly declines with prey (moose) density in Isle Royale National Park (Fig. S7a). Predation rate also has a strong influence on the growth rate of the moose population (Fig. S7b). That strong negative effect of predation rate on moose growth rates works in opposition to negative density-dependent processes (such as intraspecific competition for forage). The countervailing influence of predation on moose growth rates is apparently strong enough to result in the moose population exhibiting weakly positive density-dependent dynamics for a wide range of densities (Fig. S7c; $p = 0.01$, $df = 42$, $R^2 = 0.14$).

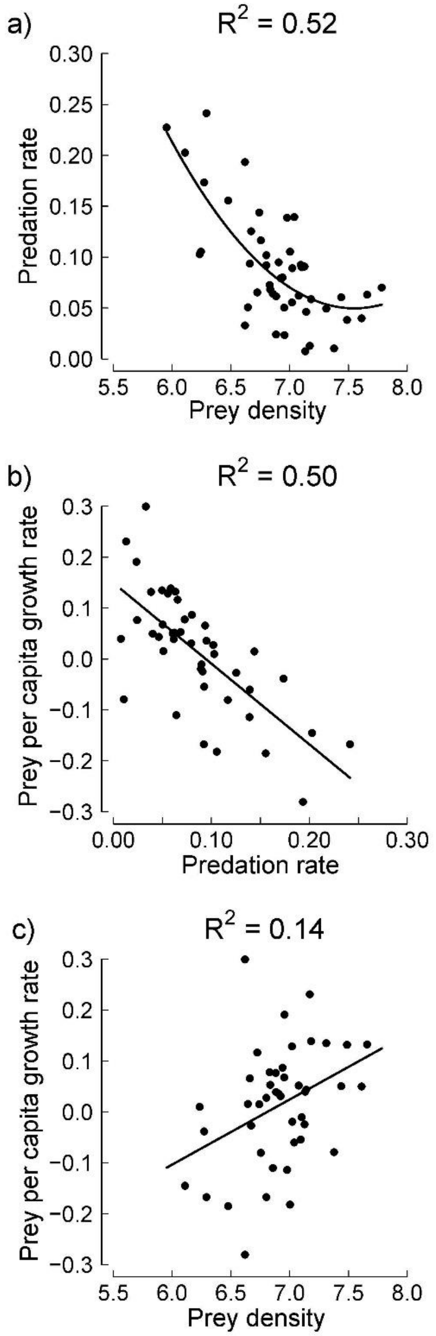


Fig. S7 The relationship between predation rate (by wolves), the per capita growth rate of the prey (moose) population, and prey density (natural log of moose abundance) for a single-predator, single-prey system, the wolves and moose in Isle Royale National Park between 1971 and 2017 (the period of time for which predation data are available). Points represent annual estimates and lines represent best fit regressions. The parsimony of the second order polynomial regression in panel (a) is supported by AIC insomuch as it performed better than liner model (ΔAIC = 5.03). We excluded three outlying data points (1996, 2007 and 2011) from the analysis of per capita growth rates (see *Statistical outliers* below for justification).

Statistical Outliers

We excluded three outlying data points (1996, 2007 and 2011) from a times series of moose population growth rates for the period 1971-2017 (n=47). We used the median absolute deviation (MAD) as a basis for quantifying a judgment about whether these observations are statistical outliers. The recommendation is to treat as statistical outliers any observation that is at least 2.5 times the MAD from the median (Leys, Ley, Klein, Bernard, & Licata, 2013; Miller, 1991). We were conservative use 3 times the MAD as a criterion. For this time series, the median growth rate is 0.04/yr and the MAD is 0.13. So, observations less than –0.34 or greater than 0.41 are statistical outliers. By this criterion, the growth rates for 1996 (– 0.62/yr), 2007 (0.69/yr) and 2011 (0.46/yr) are statistical outliers.

For additional context, estimates for r_{2007} and r_{2011} are both biologically unrealistic, insomuch as they greatly exceed the maximum intrinsic rate of increase estimated for the species, which is approximately 0.3/yr (Ruprecht, 2016). The estimates arose from estimating moose abundance in February 2008 and February 2012 during which snow conditions were not favourable for obtaining accurate estimates of moose abundance. Specifically, snow depth was well below average and shallow enough to allow moose to readily access more open habitats where they are easier to detect.

We have no reason to think that the estimate for r_{1996} (– 0.62/yr) is influenced by unusually large measurement error. However, during this year, winter severity was greater than ever recorded in the twentieth century. Additionally, moose population density was exceptionally high, and the population was afflicted with an unusually severe outbreak of winter tick. These circumstances resulted in extraordinarily high mortality. The exceptional mortality event is indicated, in part, by cohort analysis of moose necropsy data which suggests that at least 60% of the moose population died during this year (Hoy et al., 2020).

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