

Multiple nutritional currencies shape pregnancy in a large herbivore

J.R. Newby and N.J. DeCesare

Abstract: Nutritional condition embodies environmental conditions experienced by animals with survival and reproductive consequences. Body fat is often associated with ungulate fecundity; however, other nutritional currencies may limit fecundity. Using data from 129 moose (*Alces alces* (Linnaeus, 1758)) monitored over 429 moose-years, we examined the limiting role of multiple nutritional currencies on pregnancy rates while concurrently assessing the influence of age and prior reproduction. Females tended to be pregnant in successive years, suggesting differences in individual or habitat quality. However, probability of pregnancy declined with survival of calves from prior litters, indicating a reproductive cost to rearing offspring. Pregnancy was positively associated with serum iron (Fe), body fat, body mass, and serum protein. The best model predicting pregnancy included serum Fe, body fat, and age class, with serum Fe being the strongest single predictor. Moose with Fe concentrations considered deficient in cattle (*Bos taurus* Linnaeus, 1758) had pregnancy rates of 33%–35%, whereas 89%–91% of individuals with sufficient Fe were pregnant. We subsequently evaluated hypotheses concerning factors potentially limiting Fe concentrations, including Fe-deficient diet, chronic infection, parasitism, and malnutrition. The best supported hypothesis was energy and protein malnutrition constrained Fe stores. We conclude that subclinical anemia due to nutritional constraints can limit or indicate limits in moose fecundity.

Key words: *Alces alces*, fecundity, iron, moose, pregnancy, reproduction, ungulate.

Résumé : L'état nutritionnel reflète des conditions ambiantes auxquelles sont exposés les animaux qui ont des conséquences sur leur survie et leur reproduction. La masse adipeuse est souvent associée à la fécondité chez les ongulés; d'autres devises nutritionnelles pourraient toutefois limiter la fécondité. En utilisant des données de 129 orignaux (*Alces alces* (Linnaeus, 1758)) suivis sur 429 années-orignal, nous avons examiné le rôle limitatif de plusieurs devises nutritionnelles sur les taux de gestation, tout en évaluant en parallèle l'influence de l'âge et des reproductions antérieures. Les femelles avaient tendance à être gestantes lors d'années successives, ce qui indiquerait des différences sur le plan de la qualité des individus ou de l'habitat. La probabilité de gestation diminuait toutefois quand survivaient des veaux de portées précédentes, ce qui indique que l'élevage de veaux a un coût en ce qui concerne la reproduction. La gestation était positivement associée au fer (Fe) sérique, à la masse adipeuse et aux protéines sériques. Le modèle qui prédit le mieux la gestation intègre le Fe sérique, la masse adipeuse et la classe d'âge, le Fe sérique étant le prédicteur individuel le plus fort. Les orignaux femelles présentant des concentrations de Fe considérées déficientes chez les bovins (*Bos taurus* Linnaeus, 1758) présentaient des taux de gestation de 33 % – 35 %, alors que 89 % – 91 % des femelles présentant des concentrations suffisantes de Fe étaient gestantes. Nous avons ensuite évalué différentes hypothèses concernant des facteurs limitant potentiellement les concentrations de Fe, dont un régime alimentaire pauvre en Fe, des infections chroniques, le parasitisme et la malnutrition. L'hypothèse la mieux appuyée par les données est celle voulant que la malnutrition énergétique et protéique limite les réserves de Fe. Nous concluons que l'anémie subclinique causée par des contraintes nutritionnelles peut limiter la fécondité des orignaux ou indiquer la présence de limites à cette dernière. [Traduit par la Rédaction]

Mots-clés : *Alces alces*, fécondité, fer, orignal, gestation, reproduction, ongulé.

Introduction

Fecundity is a fundamental life-history component shaping population dynamics of large ungulates and other large herbivores (Gaillard et al. 2000). A broad suite of abiotic and biotic environmental factors can influence variation in fecundity among individuals, populations, and years (Sand 1996). For large ungulates, nutritional condition is a primary mediator between these factors and their ultimate effects on reproduction (Parker et al. 2009). The cost of reproduction is financed by nutritional intake and storage, and as a result, nutritional condition strongly influences reproductive output of individuals (Cook et al. 2004). Nutritional condition itself reflects the integrated effect of environmental conditions

experienced by individuals, such as climate, forage resources, intra- and inter-specific competition, and predator avoidance (Monteith et al. 2014). Therefore, assessing the interplay between nutritional condition and resulting fecundity measures can provide key insights into how habitat conditions interact with age structure and density to shape population dynamics (Solberg et al. 1999).

Nutrition comes in the form of many currencies and measures (Parker et al. 2009). Energy input and expenditure is typically considered the pre-eminent nutritional component driving animal maintenance, growth, and reproduction (Parker et al. 2009). In ungulates, body fat is the primary integrator of energy gains and demands, thus representing an important measure of nutritional condition (Parker et al. 2009; Monteith et al. 2014), and one

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with implications for reproductive output (Keech et al. 2000; Cook et al. 2004). Growth and body size across life stages is also dependent on nutrition, making body size another meaningful indicator of nutritional condition (Sand 1996; Pachkowski et al. 2013). Individual variation in body size has also been linked to differences in reproductive performance (Pachkowski et al. 2013). The effect of body size on fertility can interact with environmental conditions such that the size–fecundity relationship may strengthen under harsher environmental conditions (Sand 1996). Body size also interacts with age; particularly, the age of primiparity is dependent on early growth, making age of primiparity sensitive to nutritional conditions (Boer 1992; Gaillard et al. 2000). Though digestible protein has not received the attention digestible energy has in nutritional ecology, its importance to reproduction is well recognized (Barboza et al. 2009). This could be especially relevant in systems where plant growth is nitrogen-limited or for herbivores browsing upon plants heavily defended by secondary metabolites that reduce protein availability (McArt et al. 2009; Thompson and Barboza 2014). Protein reserves may be especially critical for pregnant females because fetal growth can require high stores of maternal body protein during winter and early spring when dietary protein is at or below minima for maintenance (Parker et al. 2009).

Beyond these focal metrics of body fat, size, and protein, micronutrients are yet other potentially limiting resources also needed for maintenance, growth, and reproduction (Barboza et al. 2009). Indeed, micronutrients, such as trace elements, are essential to a wide range of physiological processes including reproductive performance and may be more sensitive indicators of nutritional constraints on productivity (Barboza et al. 2009; Parker et al. 2009). In ruminant herbivores, mineral balance depends on interactions between geochemistry, forage plants, rumen microbes, and the physiological requirements of the animal (Van Soest 1994; Slobach et al. 2015). The impacts of deficient or imbalanced levels of trace minerals on reproductive performance are well established in livestock (Hidiroglou 1979); however, few studies have explored these relationships in wild populations (O'Hara et al. 2001).

Links between nutrition and reproductive performance can be further complicated by intrinsic differences among individuals, whether driven by maternal effects, time-lagged differences in early development, prior reproductive effort, or differences in local habitat conditions (Hamel et al. 2009; Richard et al. 2017). For iteroparous species, two contrasting predictions can arise when studying reproductive performance: (1) the costs of reproduction are such that prior reproductive success negatively affect reproductive performance in a subsequent year (Testa and Adams 1998), or (2) differences in the nutritional condition achieved by different individuals, whether driven by individual heterogeneity or local habitat conditions, lead to positive correlations between years within individuals (Hamel et al. 2009; Markussen et al. 2018).

We evaluated multiple currencies of nutrition as potential drivers of pregnancy for a large, ruminant herbivore, moose (*Alces alces* (Linnaeus, 1758)). We hypothesized pregnancy among moose would be constrained by nutrition, which we measured using multiple currencies including body size, fat stores, protein (serum albumin), and essential elements (cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)). We also evaluated the role of individual differences affecting annual reproduction by including age and prior reproductive efforts.

Materials and methods

Study system and data collection

We studied moose in three areas of western Montana, USA (Fig. 1). The mountainous Cabinet-Salish area (48.2°N, 115.5°W) was characterized by dense forest of diverse conifer species, ranging in elevations from 660 to 2494 m with mean January temperatures of –8.1 to –0.8 °C, mean July temperatures of 7.7 to 25.0 °C,

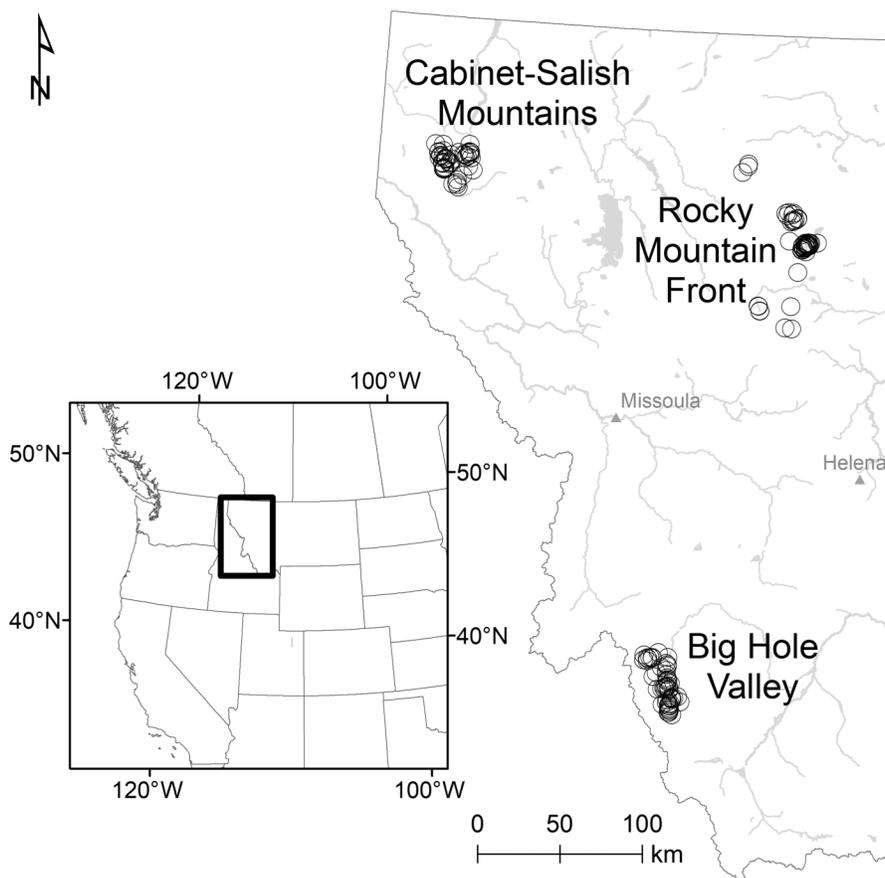
and mean annual precipitation of 91.4 cm. The Big Hole area (45.4°N, 113.5°W) was a higher elevation valley floor surrounded by forested mountains, ranging in elevation from 1842 to 3232 m with mean January temperatures of –12.0 to –2.2 °C, mean July temperatures of 5.4 to 22.9 °C, and mean annual precipitation of 63.8 cm. The Rocky Mountain Front area (47.9°N, 112.7°W) included areas of riparian and fen habitat and rugged mountainous forests, ranging in elevation from 1200 to 2803 m with mean January temperatures of –10.2 to –0.9 °C, mean July temperatures of 7.1 to 22.6 °C, and mean annual precipitation of 71.4 cm.

Between late January and early March of 2013–2018, we conducted 141 captures of female moose ≥ 1.5 years old (129 individuals) from which we collected concomitant pregnancy, age, and body condition data. All capture and handling procedures accorded with protocols approved by the Animal Care and Use Committee (permit No. FWP12-2012) of Montana Fish, Wildlife and Parks. We captured moose via helicopter darting, and we immobilized individuals with combinations of xylazine (20–50 mg/animal) and either carfentanil (3.3–3.9 mg/animal) or etorphine (8–10 mg/animal), which was reversed with tolazoline (600–800 mg/animal) and naltrexone (400–600 mg/animal), respectively. We extracted a lower canine for cementum aging (Matson's Laboratory, Milltown, Montana, USA) after injecting lidocaine into the lower mandible as a nerve block (Mansfield et al. 2006). We measured total length and chest girth of each animal and estimated body mass using the mean of estimates from regression equations relating mass to length and girth (Franzmann et al. 1978). We assessed nutritional condition by measuring the maximum depth of rump fat with ultrasonography (Micromaxx, Sonosite, Inc., Bothell, Washington, USA), which has a strong linear relationship to ingesta-free body fat, provided rump fat depth >0 ($r^2 = 0.96$; Stephenson et al. 1998). We estimated the extent of winter tick (*Dermacentor albipictus* (Packard, 1869)) parasitism using tick counts along five 10 cm transects on each of the shoulder and rump, extrapolated to estimated total tick load following Sine et al. (2009). We analyzed blood sera for seven trace minerals, total iron-binding capacity (TIBC; Michigan State University, East Lansing, Michigan, USA, and Kansas State Veterinary Diagnostic Laboratory, Manhattan, Kansas, USA), chemistry profiles (including measurement of serum albumin levels (g/dL) as a metric of protein stores; Garnier et al. 2017), and for pregnancy-specific protein B (PSPB; BioTracking, LLC, Moscow, Idaho, USA) to determine pregnancy status (Huang et al. 2000). In addition, complete blood counts were obtained from whole blood samples for 71 moose captured during 2014–2016. We also collected fecal samples during capture as a second method of assessing pregnancy status based on fecal progesterone concentrations (ng/g dry mass; Smithsonian Conservation Biology Institute, Front Royal, Virginia, USA; Murray et al. 2012).

We fit captured moose with either VHF (LMRT-4; Lotek Wireless Inc., Newmarket, Ontario, Canada) or GPS (LifeCycle (Lotek Wireless Inc., Newmarket, Ontario, Canada) or Survey Globalstar (Vectronic Aerospace, Berlin, Germany)) radio collars to document parturition and subsequent calf survival, and to monitor pregnancy in subsequent years. Each year during the calving season (12 May – 16 June), we conducted weekly aerial telemetry of collared moose to determine timing of parturition. We then monitored calf survival during the first 10–11 months of life using aerial telemetry. In addition, we conducted ground-based telemetry to further ascertain calf status.

During subsequent winters following an initial capture event, we monitored pregnancy of individuals using fecal progesterone measurements from fecal samples collected January–April during ground-based radio-tracking of free-ranging, collared females. We used visual observations and snow tracks to ensure fecal samples were from the target animal, and stored samples frozen until submission. In cases when we could not attribute a given fecal sample to the target individual with certainty, we swabbed the mucous membranes of pellets during collection for genetic analysis at the

Fig. 1. Study areas and capture locations of 141 captures of 129 female moose (*Alces alces*) in Montana, USA, from 2013 to 2018. Map created using ArcGIS Desktop version 10.6.1 (ESRI, Inc., Redlands, California, USA) and includes base-map data provided by the Montana Spatial Data Infrastructure (MSDI; available from <http://geoinfo.msl.mt.gov/msdi> and reproduced with permission of the Montana State Library).



National Genomics Center for Wildlife and Fish Conservation (Missoula, Montana, USA). Identity of individuals for these samples were verified through analysis of 13 microsatellite markers from fecal DNA for comparison with genetic signatures from whole blood samples collected at the time of capture (Brinkman et al. 2010).

Data analysis

We used a combination of data sources to document pregnancy status each winter, including PSPB assays of blood sera, fecal progesterone concentrations, and visual observation of calves at heel during the calving season. We developed a set of ordered criteria for final determination of pregnancy status among multiple data streams for each animal-year (Supplementary text).¹ Additionally, we used logistic regression to develop a predictive model for determining pregnancy status from fecal progesterone results (ng/g) according to paired fecal progesterone and PSPB samples collected concurrently during animal captures (Supplementary eq. S1).¹ In short, fecal progesterone concentrations ≥ 853 ng/g were assigned a positive pregnancy status, and the model showed excellent ability to discriminate between pregnant and non-pregnant females (area under the receiver operating characteristic curve (AUC) = 0.962) (Supplementary Fig. S1).¹

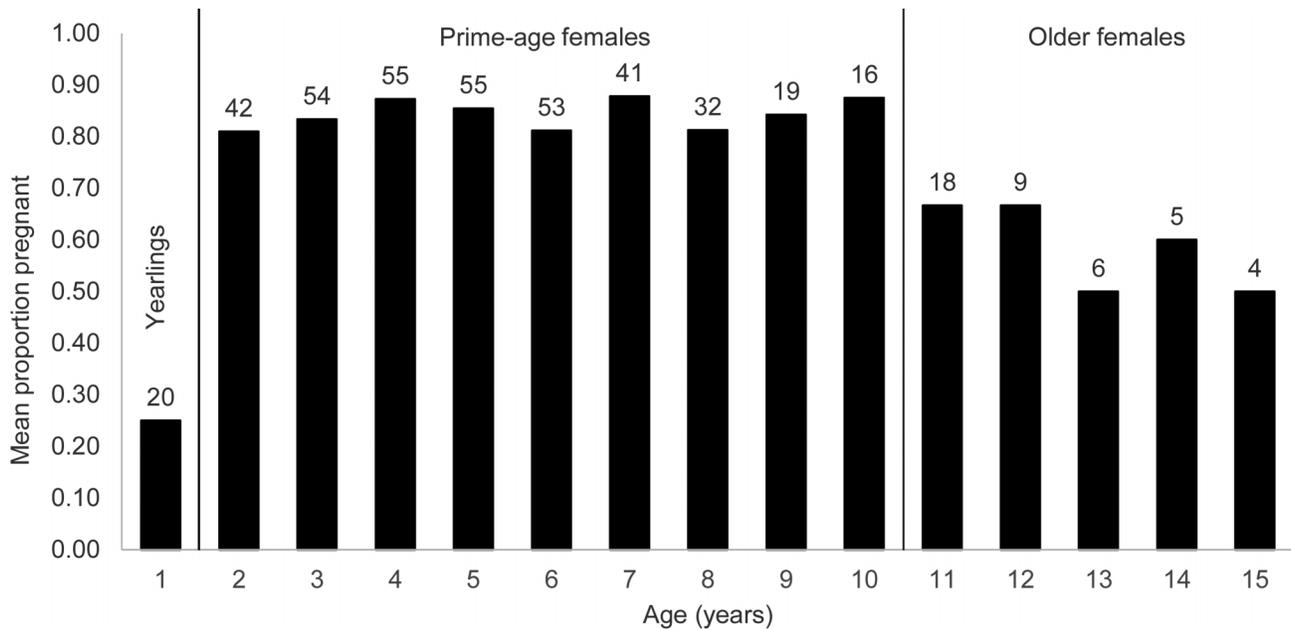
We then used two data sets to assess potential drivers of moose pregnancy status. The smaller, nutrition-focused data set ($N = 141$) included a suite of nutritional currency information requiring the

capture and handling of moose (e.g., blood and fat assays). The larger longitudinal data set included subsequent years of monitoring after capture, but was limited only to covariates of age ($N = 429$ moose-years) and prior reproduction ($N = 306$ moose-years). We began our analyses using the longitudinal data set to assess the influence of dam age and prior reproductive efforts on pregnancy during a given year. We then used results from this analysis to identify variables to be included during subsequent evaluation of nutritional currencies as drivers of pregnancy status.

To evaluate the effects of age on pregnancy rates, we visually inspected the proportion of females pregnant in each year of life (1–16 years old) and developed several competing alternatives for grouping ages into age classes (Fig. 2). Competing models included the following: (i) null, no age effects on pregnancy; (ii) yearling, adult (>2 years old); (iii) yearling, adult (2–10 years old), older adult (>10 years old); (iv) age-specific, with each age categorized separately. We then evaluated potential positive or negative effects of prior reproductive investment on pregnancy status. Models of prior reproductive effects on current reproduction included combinations of the following: (i) pregnancy status in previous year (0, 1); (ii) litter size in previous year (0, 1, 2 calves); (iii) days with prior calf at heel (0–365 days); (iv) success in raising the calf from previous years to a yearling (0, 1). Finally, we evaluated spatio-temporal effects by including study area and year in candidate models. Timing of captures and associated sampling varied between

¹Supplementary materials (text, Fig. S1, eq. S1, Table S1) are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2019-0241>.

Fig. 2. Proportion of 1- to 15-year-old female moose (*Alces alces*) pregnant during winter, with sample sizes above each bar, in Montana, USA, from 2013 to 2018. The denoting grouping of yearling, adult (2–10 years old), and older adult (>10 years old) was most supported during data analysis.



January and March between years, potentially influencing both detection of pregnancy and body fat measurements. Therefore, we also used an exploratory linear regression analysis to test for an effect of Julian capture day on body fat and found it did not significantly affect our data ($P = 0.295$).

After accounting for the above parameters, we then evaluated multiple currencies of nutritional condition as putative correlates to pregnancy in moose using the smaller ($N = 141$) capture-based data set. These included female body mass (kg), the presence (0, 1) and depth (cm) of rump fat, serum albumin levels (g/dL) (as indicator of protein stores; Garnier et al. 2017), and seven trace elements (concentrations of Co ($\mu\text{g/mL}$), Cu ($\mu\text{g/mL}$), Fe ($\mu\text{g/dL}$), Mn (ng/mL), Mo (ng/mL), Se (ng/mL), and Zn ($\mu\text{g/mL}$)). Potential non-linear relationships between explanatory variables and pregnancy were explored by viewing histograms of explanatory variable data and fitting transformed data into generalized linear models of pregnancy status. No transformed variables improved model performance as measured by Akaike's information criterion corrected for small sample size (AIC_c) values. No variables with ≥ 0.6 correlation were included in the same model.

Although we could not document prior pregnancy status or days with a calf at heel during capture events, we did record the presence or absence of calves at heel during captures. Inclusion of the binary variable of calf presence at time of capture did not improve models within this smaller capture-based data set. Therefore, we did not include a covariate for prior reproductive history in subsequent models assessing nutritional effects.

We evaluated all variables using generalized linear models with pregnancy status as the response variable and a logit link function. We used supervised forward-selection procedures, following Hosmer et al. (2013). We compared models of demographic variables (age, study area, year, prior reproduction) on pregnancy using AIC_c Burnham and Anderson 2002) to ensure demographic effects predictive of pregnancy were accounted for in subsequent model testing. We then followed the same procedures to identify nutritional currencies limiting pregnancy rates with the smaller capture-based data set. We used AIC_c to compare the relative support for models, including estimation of AIC_c weights (w_i) for each model (i). Once a final model was selected, we used hierarchical partitioning to measure the relative independent effects of nutri-

tional covariates associated with pregnancy using the hier.part package in R (Walsh and Mac Nally 2017).

We evaluated the final selected model's capacity to discriminate between pregnant and non-pregnant individuals using the Hosmer-Lemeshow test and the AUC (Hosmer et al. 2013). We further evaluated the predictive accuracy of the model using k -fold cross-validation ($k = 5$) and estimated prediction error adjusted for upward bias associated with $k < n$ cross-validation (Fushiki 2011).

Assessing factors influencing Fe stores in female moose

The above analyses revealed Fe to be an important nutritional currency shaping moose pregnancy. In response, we conducted additional post hoc analyses to better understand the relationships between serum Fe and other health metrics. Review of this topic in the scientific literature indicated four hypotheses (H1–H4) to explain the reduced Fe levels in non-pregnant females: (H1) diets deficient in Fe, (H2) chronic infection, (H3) parasitism, and (H4) constrained uptake of Fe related to nutritional condition.

We developed a suite of predictions from these four hypotheses. First, diets deficient in Fe (H1) should manifest as microcytic, hypochromic anemia, which is typical in humans but rare in ruminants (Borelli et al. 2007). Animals provided adequate dietary Fe should exhibit serum Fe levels positively correlated with TIBC. In contrast, with Fe-deficient diets, a shortage of Fe ions is available to bind to transferrin and the correlation between Fe levels ($\mu\text{g/dL}$) and TIBC ($\mu\text{g/dL}$) would be lost or even negative. We tested the dietary Fe hypothesis by estimating the correlation between serum Fe and TIBC, considering the hypothesis falsified if these variables were positively correlated.

Second, low serum Fe may be due to sequestration as an immune response to chronic infection (H2), which is considered the most common cause of low serum Fe levels among mammals (Passler et al. 2013; Baydar and Dabak 2014). Under this hypothesis, we predicted Fe levels should correlate negatively with other common indicators of infection, including fibrinogen (mg/dL), neutrophils (1×10^9 neutrophils/L), and neutrophil to lymphocyte ratios (Passler et al. 2013).

Third, anemia might be induced by chronic blood loss due to parasitism (H3). Tick epizootic events have been associated with

anemia in moose and may ultimately reduce pregnancy (Jones et al. 2019). Moose are also susceptible to severe giant liver fluke (*Fascioloides magna* Bassi, 1875) infection, resulting in blood loss and anemia (Murray et al. 2006). If internal or external parasites are consistently leading to anemia, then we predicted that red blood cell (RBC) counts (1×10^{12} RBC/L) should be positively related to Fe levels, since blood loss is thought to be the primary mechanism by which parasitism causes anemia (Jones et al. 2019). Covariates selected to reflect the severity of parasitism-induced blood loss included RBC count, estimated winter tick count at time of capture, and elevated aspartate aminotransferase (AST; UI/L) and gamma-glutamyltransferase (GGT; UI/L) enzyme levels indicative of liver damage (Lotfollahzadeh et al. 2008).

Fourth, constrained Fe absorption may occur due to caloric or protein malnutrition, leading to anemia (H4; Sood et al. 1965; Furugouri 1984). If Fe levels are impacted by the ability to acquire and maintain stores of energy and (or) protein, then we predicted that Fe should be positively related to rump fat depth (cm) and serum albumin (g/dL), respectively. Estimated mass was also included as an additional indicator of nutritional condition, along with an interaction between mass and female age. To account for the possibility that young animals exhibited physiological anemia during growth, we also included a binary covariate for moose age (yearling, adult (>2 years old)) in all models used to test these four hypotheses (Rostal et al. 2012).

Measures of some explanatory variables relevant to the above hypotheses were only available for a subset of captured individuals. To maximize effective use of limited samples, we used a hypothesis-driven approach to analysis. We first identified the best model characterizing each hypothesis by developing competing models for each hypothesis incorporating the suite of associated explanatory variables. The subset of individuals for which all relevant covariate measurements were available under a given hypothesis were used to test competing linear regression models. Supervised forward selection based on AIC_c were used to identify the most parsimonious model. Once the best model was identified within each of these four hypotheses, we again used linear regression and w_i to compare support among hypotheses, as well as to assess support for combinations of hypotheses. The final model represented the best single hypothesis or combination of hypotheses in explaining variation in Fe levels in moose.

Results

During 2013–2018, we obtained concurrent age, pregnancy, and condition data from 141 captures of 129 individual female moose. The distribution of captures across study areas was 46 in the Cabinet-Salish, 50 in the Big Hole, and 45 in the Rocky Mountain Front study areas. Additional reproductive data were collected as we followed moose over subsequent years (26 moose monitored for 2 years, 16 moose for 3 years, 23 moose for 4 years, 19 moose for 5 years, and 17 moose for 6 years). In total, we determined pregnancy status and age for 429 moose-years across all animals and years of monitoring. Mean age at time of capture in the Cabinet-Salish was 7.3 years (SD = 3.7 years), in the Big Hole was 5.4 years (SD = 3.1 years), and on the Rocky Mountain Front was 3.9 years (SD = 2.4 years).

Factors influencing moose pregnancy

Age and prior reproductive effort

Pregnancy rates averaged 81.9% for adults >2 years old ($N = 409$ moose-years) and 25.0% for yearlings ($N = 20$ moose-years) (Fig. 2). Age breaks of yearling, adult (2–10 years old), and older adult (>10 years old) were best supported among generalized linear models ($w_i = 0.986$). We used these three age categories in all subsequent models to account for age effects on pregnancy status. Models predicting pregnancy status were not improved by includ-

ing effects for study area or year. Therefore, data were pooled across years and study areas in subsequent model comparisons.

We found both positive and negative relationships between prior reproductive efforts and current pregnancy status of adult (>2 years old) moose (Fig. 3A). The best supported model of past reproductive effects ($N = 306$ moose-years) included age (older age class, $\beta = -1.393 \pm 0.0502$), a positive effect of prior pregnancy status ($\beta = 1.406 \pm 0.056$), but a negative effect of the number of days with one or more calves at heel ($\beta = -0.003 \pm 0.0001$).

Nutritional constraints

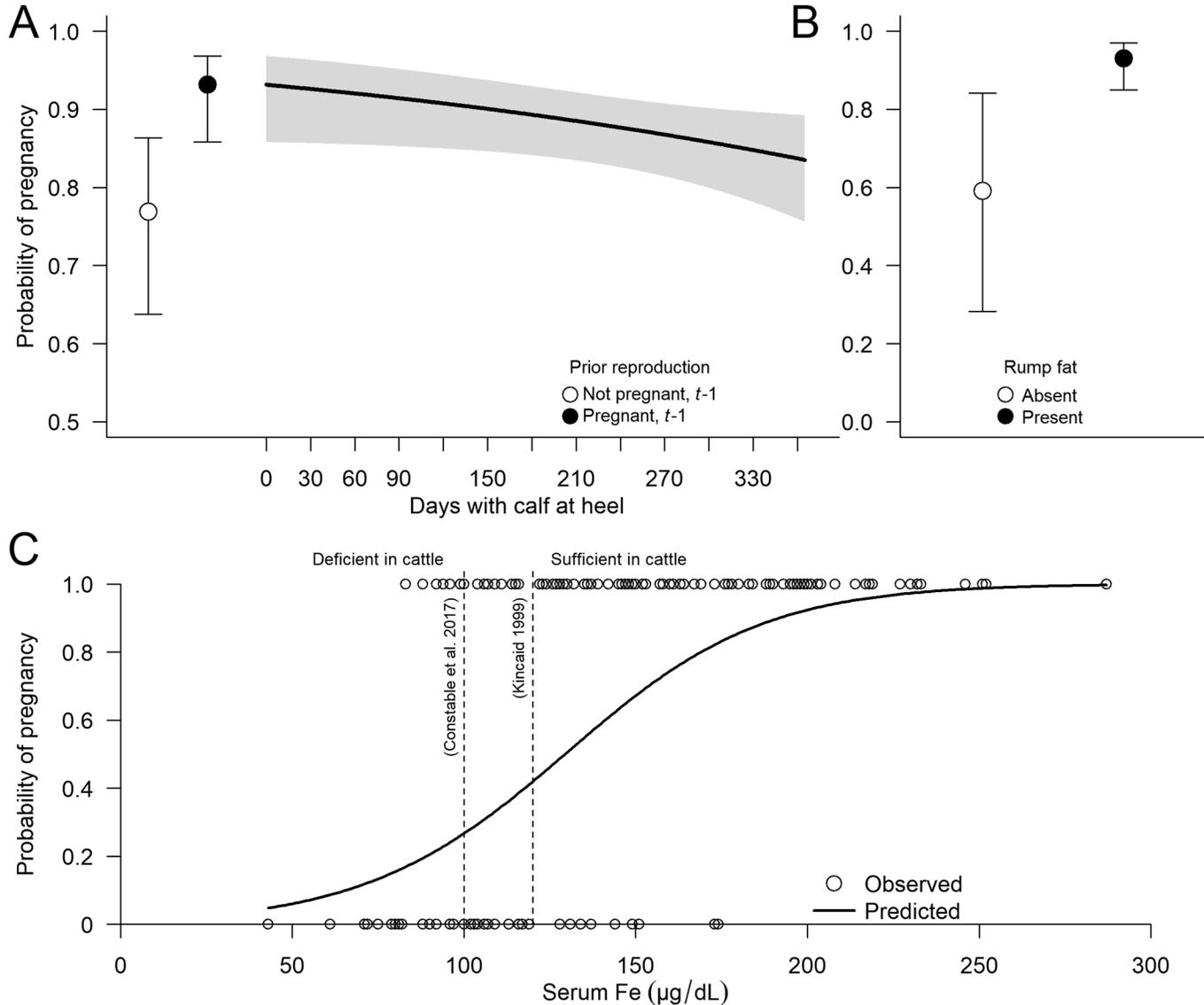
We assessed the potential for moose pregnancy to be limited by multiple nutritional currencies based on pregnancy status and nutritional condition measures obtained during winter captures ($N = 141$). We found no evidence of a relationship between pregnancy status and trace mineral levels of Co, Cu, Mn, Mo, Se, or Zn. Covariates that did show positive univariate effects on pregnancy status included female mass, rump fat presence, rump fat depth, serum albumin, and serum Fe (Table 1). No interaction terms between these covariates and age were found to improve model performance. The two strongest predictors of pregnancy were serum Fe ($\beta = 0.036 \pm 0.0015$) and the presence of rump fat ($\beta = 2.223 \pm 0.1078$; Fig. 4), and the best multivariable model included the effects of age and these two nutritional currencies (Table 1). The Hosmer–Lemeshow test showed the final model to be well calibrated ($\chi^2 = 3.25$, $df = 8$, $P = 0.918$), and the AUC indicated excellent ability to discriminate between pregnant and non-pregnant moose (AUC = 0.929). Similarly, k -fold cross-validation indicated the model had a low error rate in predicting pregnancy status (adjusted error rate = 0.114).

Maximum rump fat depth measured on adult female moose during winter captures ranged from 0.0 to 3.35 cm (mean = 0.82 cm). However, the presence versus absence of rump fat better predicted moose pregnancy than the continuous measure of fat depth. Comparison between females with and without rump fat showed the odds of pregnancy for females with rump fat were approximately 9.2 times higher than those without (Fig. 3B). Serum Fe measurements from captured moose ranged from 41 to 287 $\mu\text{g/dL}$ (mean = 149 $\mu\text{g/dL}$). All moose with Fe values <83 $\mu\text{g/dL}$ were non-pregnant ($N = 14$) and all moose with Fe values >174 $\mu\text{g/dL}$ were pregnant ($N = 50$). Based on the univariate model, Fe values ≥ 108 $\mu\text{g/dL}$ corresponded to ≥ 0.5 probability of pregnancy (Fig. 3C).

Assessing factors influencing Fe stores in female moose

In testing hypothesized factors influencing Fe stores, we initially evaluated models for each hypothesis separately using all samples available for their respective covariates. A model of moose serum Fe concentrations that distinguished yearling and adult moose improved AIC_c scores over the null model ($N = 153$, $\Delta AIC_c = 8.3$); therefore, an age effect was included in all subsequent models. Following hypothesis H1, that Fe stores in moose were limited by Fe-deficient diets, we predicted a lack of Fe ions to bind with transferrin (e.g., a lack of positive correlation between Fe and TIBC). Linear regression models supported a positive relationship between Fe stores and TIBC ($N = 116$, $\beta = 0.35 \pm 0.0118$); therefore, we rejected the Fe-deficient diet hypothesis and we did not further consider this hypothesis in subsequent comparisons. All models representing hypothesis H2, that Fe was limited by chronic infection, were supported, but the best performing model included only fibrinogen levels and age effect ($N = 65$, $w_i = 0.659$). Hypothesis H3, that blood loss due to parasitism was limiting Fe stores, was not supported. RBC counts were not strongly related to Fe levels and inclusion of other indices of parasitic infection (GGT, AST, and tick counts) did not improve models ($N = 63$). Therefore, we did not include parasitism in subsequent comparisons between alternative hypotheses. We evaluated support for hypothesis H4, that nutritional condition limits Fe stores, with covariates for energetic (rump fat) and protein (serum albumin) nutritional

Fig. 3. (A) Probability of adult (>2 years old) female moose (*Alces alces*) pregnancy in current year (t) as a function of being not pregnant (open circle) or pregnant (solid circle) during the previous year ($t-1$), as well as the estimated decline in probability of pregnancy among previously pregnant females as a function of the number of days a calf survived, in Montana, USA, from 2013 to 2018. (B) Probability of adult female moose pregnancy and 95% confidence intervals for individuals with and without measurable rump fat during winter. (C) Observed moose pregnancy status (open circles) and predicted probability of moose pregnancy (solid line) relative to serum iron (Fe) concentrations ($\mu\text{g}/\text{dL}$). Vertical broken lines indicate serum Fe concentrations below which domestic cattle (*Bos taurus*) are considered Fe deficient, based on two sources (Kincaid 1999; Constable et al. 2017).



status, along with an interaction between age and body mass. An effect of moose mass was not supported. However, both rump fat and serum albumin were positively related to Fe stores and the best model ($N = 141$, $w_i = 0.978$) included these two nutritional currencies (Figs. 5A and 5B). Final model comparisons across these four hypotheses indicated that nutritional condition (H4), both energetic and protein, was best supported ($N = 62$; Supplementary Table S1).¹ Models including measures of body fat and protein outperformed the null model (age only), the chronic infection model (H2), and a combined model of both nutritional condition and chronic infection (H2 + H4).

Discussion

We identified multiple nutritional currencies strongly associated with reproductive performance of moose in western Montana while accounting for differences in female age and reproductive history.

Specifically, we found evidence moose pregnancy was associated with age, prior reproductive effort, female body mass, protein stores, and especially, body fat; however, serum Fe was the strongest predictor of moose pregnancy status. Fe is an essential element in hemoglobin, along with a broad range of cellular processes, and demands are especially high during pregnancy for fetal development (Barboza et al. 2009). Despite the high fetal demand for Fe during pregnancy, our results showed a positive relationship between moose pregnancy and serum Fe stores of a female. This suggests the possibility for Fe deficiency to limit moose reproduction, an outcome that has been demonstrated in studies of domestic ruminants (Hidirolou 1979; Modi et al. 2013). We tested multiple hypotheses to explain such limitations in observed Fe stores that results in less fecund moose. Although inflammation and parasites have commonly been implicated in anemia (Passler et al. 2013; Baydar and Dabak 2014) and tick loads

Table 1. Model selection results comparing the number of parameters (k) and Akaike's information criterion corrected for small sample size (AIC_c) among models of the influence of nutritional currencies on pregnancy rates among 141 captures of 129 female moose (*Alces alces*) in three study areas in Montana, USA.

Model description	k	AIC_c	ΔAIC_c	w_i
Age class + Fe + rump fat	5	93.2	0.0	0.984
Age class + Fe + mass	5	102.1	8.9	0.011
Age class + Fe	4	103.9	10.7	0.005
Age class + rump fat	4	115.1	22.0	0.000
Age class + mass	4	143.7	50.5	0.000
Age class + albumin	4	144.4	51.2	0.000
Age class	3	149.9	56.8	0.000

Note: Variables include serum iron (Fe; $\mu\text{g/dL}$), serum albumin (g/dL), presence of rump fat (0 or 1, where 0 is absent and 1 is present), estimated mass (kg), and age class (yearling, 2–10 years old, and >10 years old). Models that did not improve AIC_c scores over the model with age class only are not shown. w_i is AIC_c weight.

have been associated with anemia in moose (Jones et al. 2019), we found no evidence that tick loads significantly reduced Fe levels or pregnancy. It is possible that tick infestations in our study areas were not severe enough to significantly affect Fe levels. Instead, Fe was most associated with the protein status and body fat of females, supporting the hypothesis that malnutrition was impacting Fe absorption. Both protein and caloric malnutrition interferes with Fe absorption and have been documented as leading to anemia in mammals (Sood et al. 1965; Bowering et al. 1977; Furugouri 1984). Interestingly, the effects of Fe on pregnancy appear to be, at least partially, independent of its collinearity with body protein and fat.

Serum Fe may prove a useful nutritional currency for monitoring ungulates if the patterns found here are consistent across other taxa and locations. Reference serum Fe levels have not been established for moose, but ranges observed in Montana moose (mean = 154 $\mu\text{g/dL}$, SD = 50 $\mu\text{g/dL}$) were similar to those reported from New Hampshire (mean = 154 $\mu\text{g/dL}$, SD = 31 $\mu\text{g/dL}$; Jones 2016) and Alaska (mean = 161 $\mu\text{g/dL}$, SD = 64 $\mu\text{g/dL}$; Franzmann and Schwartz 1983). In domestic cattle (*Bos taurus* Linnaeus, 1758), reference levels vary at which Fe is reported to be deficient. Kincaid (1999) considered serum Fe concentrations $\leq 120 \mu\text{g/dL}$ deficient in cattle, whereas Constable et al. (2017) considered serum Fe concentrations $\leq 100 \mu\text{g/dL}$ to be deficient. Using the above values as proxies for moose in Montana, pregnancy rates for adults (>2 years old) with serum levels considered deficient in cattle were 33%–35%, whereas pregnancy rates for adults with adequate Fe concentrations were 89%–91%.

Regardless of the reference value used, our findings corroborate those of Jones (2016) who found significantly higher Fe levels in pregnant versus non-pregnant moose and in moose with calves in spring versus those without. However, some cautions are warranted should Fe be interpreted as an index of nutritional condition relevant to fecundity in other systems. Additional sampling variation in Fe levels may be caused by multiple factors: including natural temporal variation in serum concentrations, hemolysis of samples, and a range of physiological factors that we were unable to assess (Barboza et al. 2009). Measures of hepatic Fe from liver biopsies of harvested animals may be less sensitive to some of these sampling concerns (Kincaid 1999). Research specifically designed to test mechanisms shaping Fe stores in wild ungulates, and how this may limit fecundity, are needed to better understand the pregnancy–Fe relationship that we documented.

Typically, large ungulate life cycles show pregnancy rates that are low and variable at young ages, consistently high for prime-age females, and marginally lower or more variable for older females (Gaillard et al. 2000). Moose in our study areas exhibited this pattern, with low yearling pregnancy followed by subsequent

high pregnancy until approximately 10 years old. This pattern is very similar to other moose populations in North America (Ruprecht et al. 2016) and Scandinavia (Ericsson et al. 2001; Markussen et al. 2018). Studies of moose elsewhere have shown yearling pregnancy rates to closely indicate population-level nutritional condition (Boer 1992; Boertje et al. 2007). In our case, the pooled mean yearling pregnancy rate of 25% falls below mean values from North America (49%) and Sweden (31%), and below the 32% observed in a previous study elsewhere in Montana (Schladweiler and Stevens 1973; Sand and Cederlund 1996). This may indicate nutrition is playing a limiting role in our study populations (Boer 1992), which is a conclusion also supported by low twinning rates in these Montana populations (Ruprecht et al. 2016; Allen et al. 2017).

We found evidence that prior reproductive efforts influenced moose pregnancy in complex ways, which is reflective of the mixed results from other studies (Sand 1996; Testa 2004; Murray et al. 2006; Markussen et al. 2018). Similar to other studies, adult moose pregnant in one year were more likely to become pregnant the following year in our study areas. This autocorrelation in reproductive performance may be due to variation in individual or habitat quality (Hamel et al. 2009; Richard et al. 2017; Markussen et al. 2018). In contrast, after accounting for the positive autocorrelation in pregnancy, the subsequent length of time spent rearing calves reduced the probability of pregnancy. This suggests costs of reproduction accrued with successful rearing of calves. Although a negative trade-off between current and future reproduction has been documented in moose (Testa and Adams 1998), other studies have found no evidence that calf rearing reduced subsequent reproduction (Allen et al. 2017; Markussen et al. 2018). Potentially the costs of calf rearing may vary depending on the environment in which it occurs and the demands it places on females with young (Richard et al. 2017).

Past research on moose and other ungulates has found reproduction to be dependent on salient measures of nutritional condition, including body mass, protein, and fat. Among Montana moose populations, we also found positive relationships between reproduction and body mass, protein stores (i.e., serum albumin), and rump fat. Body mass can interact with age class and environmental conditions to influence ungulate reproduction (Sand 1996; Gaillard et al. 2000; Monteith et al. 2014). McArt et al. (2009) found that differences in reproductive productivity corresponded to available protein in browse species. In late winter, protein reserves may be especially limiting as demands for fetal growth are high and protein content of available in forage is low (Parker et al. 2009). In addition, as winter body fat reserves become exhausted, animals are less able to spare body protein as an energy source (Garnier et al. 2017). The usefulness of body fat as a nutritional currency likely stems from the strong association that fat accretion has with digestible energy in forage and its ability to track nutritional intake and demands (Cook et al. 2010; Monteith et al. 2014). For capital breeders such as moose, fat dynamics may be especially important to reproduction (Keech et al. 2000). Our study mirrored ungulate research showing rump fat, as an indicator of body fat stores, to be an important predictor of moose pregnancy (Stephenson et al. 1998; Cook et al. 2010). For moose in our study areas, rump fat was second only to serum Fe in its influence on pregnancy. Furthermore, the observation that Fe pools themselves were partially dependent on rump fat attests to the potential for caloric nutrition to limit reproduction through multiple pathways.

In summary, animal condition integrates the resource demands imposed by their environment, and this has demographic consequences (Parker et al. 2009; Monteith et al. 2014). Various currencies of nutritional condition have been shown to constrain ungulate reproduction, including body mass, body protein, and body fat (Gaillard et al. 2000; Cook et al. 2010). Pregnancy in Montana moose populations showed evidence of being influenced by these nutritional currencies, especially body fat, but pregnancy

Fig. 4. Relative importance of age and nutrition covariates influencing moose (*Alces alces*) pregnancy, based on hierarchical partitioning of variance in Montana, USA, from 2013 to 2018.

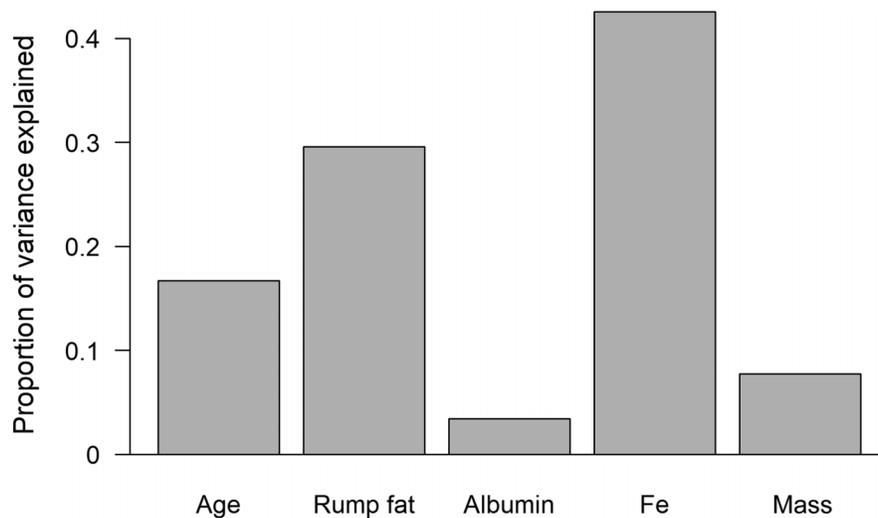
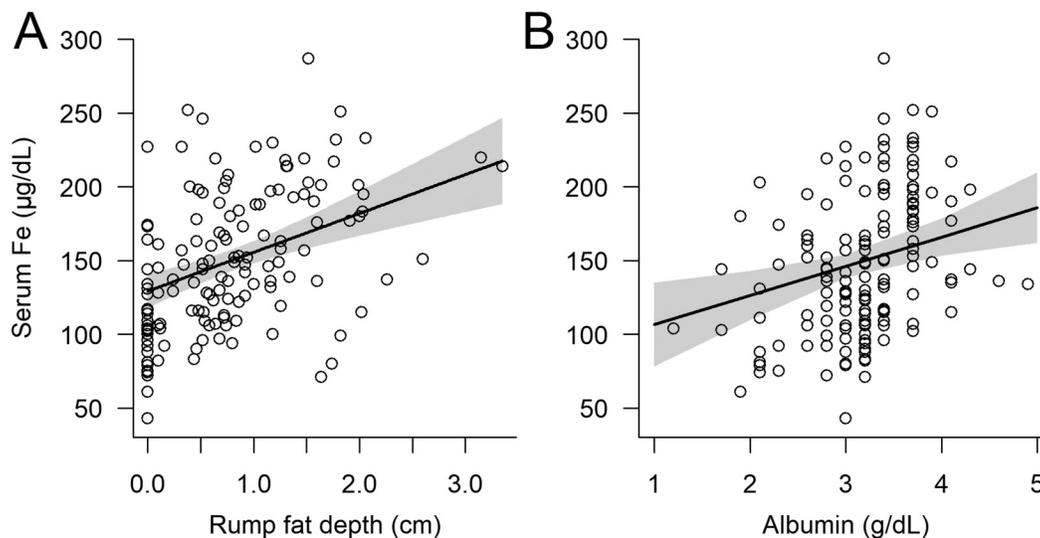


Fig. 5. Observed values (open circles) and predicted relationships (lines) with 95% confidence interval (shading) from the best model, with other variables in the model fixed at their means, relating female moose (*Alces alces*) serum Fe concentrations ($\mu\text{g/dL}$) with (A) rump fat depth (cm) and (B) serum albumin concentrations (g/dL) in Montana, USA, from 2013 to 2018.



was most strongly associated with Fe pools. This suggests that anemia owing to poor Fe absorption and uptake can constrain, or indicate constraints in, fecundity.

Ethical standards

All applicable institutional and (or) national guidelines for the care and use of animals were followed.

Conflict of interest statement

We declare that we have no conflict of interest.

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