AQUATIC AREAS PROVIDE HIGH NITROGEN FORAGE FOR MOOSE (*ALCES ALCES*) IN ISLE ROYALE NATIONAL PARK, MICHIGAN, USA

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ABSTRACT: The distribution of ungulates reflects spatial and temporal heterogeneity in forage quality and quantity across the landscape. Aquatic habitats have a patchy spatial distribution and are readily used by moose (*Alces alces*) and other ecotone specialists. However, the importance of aquatic feeding to moose has largely been attributed to acquisition of sodium, with little consideration given to the relative and comparative quality of aquatic and terrestrial forage types. We show differences in forage quality as measured by crude protein content and carbon:nitrogen (C:N) ratios between aquatic and terrestrial summer forage in Isle Royale National Park, Michigan, USA. Aquatic macrophytes had higher crude protein content and lower C:N ratio than preferred terrestrial plant species of moose. Consequently, measurable consumption of aquatic forage may provide high quality forage in less than optimal habitats. Because the distribution of aquatic habitats on Isle Royale exhibits strong spatial trends, the benefits of aquatic feeding may have spatial influence on the population dynamics of Isle Royale moose.

Ecotone specialists that consume both terrestrial and aquatic resources link food webs between these two realms (Bartels et al. 2012, Severud et al. 2013, Johnston 2017, Bump 2018). The patchy spatial and temporal distribution of aquatic cover types, and the quality and quantity of forage contained therein, are important factors in predicting the landscape distribution and density of ecotone specialists (Crawley 1983, McNaughton 1988, Fryxell 1991, Wallis DeVries 1996, Johnston and Windels 2015). Indeed, moose (*Alces alces*) link aquatic and terrestrial biomes due to their extensive foraging activities in both habitats during summer (Peterson 1955, Qvarnemark and Sheldon 2004, Peek 2007, Tischler et al. 2019).

Moose are restricted to northern latitudes characterized by a high degree of seasonal variability (e.g., short growing season) in forage quality and quantity (Timmermann and Rodgers 2017). Due to the low quality of winter forage, moose are in negative energy balance during winter and rely upon energy reserves attained during late summer and autumn to maintain energy balance year-round (DelGiudice et al. 1997, 2011, Moen et al. 1997, Schwartz and Renecker 2007). To maximize these reserves, moose face a trade-off between the benefits of exploiting high quality forage patches with potential costs of predation risk (Edwards 1983), high ambient air temperature (Renecker and Hudson 1986, 1990), and insect avoidance

(Renecker and Hudson 1990). As a result, moose exhibit distinctive habitat use patterns including summer foraging in aquatic habitats.

Aquatic habitats are typically abundant across boreal landscapes and readily used by moose in summer (Peterson 1955, Qvarnemark and Sheldon 2004, Peek 2007). Moose also use deeper water to protect against predation (Mech 1966, Gasaway et al. 1983, Jordan et al. 2010) and seek relief from high ambient temperatures (Renecker and Hudson 1986, 1990). The quality of aquatic forage, in terms of available energy, protein, and essential nutrients, has been the focus of many studies and some debate (Botkin et al. 1973, Fraser et al. 1980, 1984, Jordan 1987). Due to its scarcity in continental ecosystems, sodium may be a limiting nutrient for North American herbivores (Hutchinson and Deevey 1949), and Belovsky (1981) and Jordan (1987) noted the high sodium content of aquatic macrophytes. Several investigators have advanced the hypothesis that moose seek aquatic habitats explicitly to satisfy sodium requirements (Hutchinson and Deevey 1949, Jordan et al. 1973, Belovsky 1981, Fraser et al. 1984, Jordan 1987). However, sodium was not considered the predominant factor for moose consuming emergent aquatics on the Copper River Delta, Alaska. Rather, MacCracken et al. (1993) considered that the impetus for aquatic foraging in some systems was that aquatic forage was nutritious, high in digestible energy and crude protein. However, few studies have assessed the overall nutrition, protein, and energy associated with aquatic forage for moose, and importantly, as compared with terrestrial forage (but see Fraser et al. 1984 and MacCracken et al. 1993).

Our goal was to measure and compare the relative nutritional value of aquatic and terrestrial moose forages as measured by crude protein content and carbon:nitrogen (C:N) ratios at Isle Royale National Park, Michigan, USA. We predicted that aquatic macrophytes contain more protein and have lower C:N ratios than terrestrial plants. In this system, sodium is a seasonally important nutrient for moose (Jordan 1987), with aquatic habitats dominated by submergent (plants either free-floating or entirely submerged beneath water surface) rather than emergent (plant parts emergent above water surface) species. Apart from vegetation, sodium is also available at mineral licks and springs on Isle Royale which may be in sufficient abundance to meet the nutritional needs of moose given that these sources are well-used by moose (Risenhoover and Peterson 1986). We additionally compared forage quality among plant species composing the principal terrestrial summer diet of moose on Isle Royale, and evaluated spatial differences in forage quality between eastern and western sides of the island due to different glacial history (Huber 1973; see Study Area).

STUDY AREA

Isle Royale is a 544 km^2 island archipelago located in the boreal forest region of northwestern Lake Superior, USA, 24 km from the nearest shoreline (48° N, 89 °W). The primary island consists of Precambrian-aged basalt and conglomerate bedrock shaped by the last glaciation into a series of parallel ridges and valleys including numerous water bodies (Huber 1973). Lakes and ponds (n = 84 ≥ 1 ha) comprise 36 km^2 of the surface area, with an additional 8 km^2 of palustrine emergent wetlands. Additional shoreline is found in numerous bays of Lake Superior, particularly at the east end of the island.

As a result of glacial activity, soils are more developed on the west end of Isle Royale (Huber 1973). Fire has historically burned the entire east end, and relatively recent fires (1936 and 1948) have burned the

midsection of the island. Moose have been on Isle Royale since the early 1900s, with winter density ranging from 1 to 4 moose/km^2 across the island in the past ~50 years (Vucetich and Peterson 2004). The legacy of the island's disturbance regime has resulted in forest succession following different trajectories on the east and west ends of the island. Forests on the west end are in a late successional stage and dominated by deciduous species, while forests on the east end are younger and conifer-dominated (Janke et al. 1978).

Mean daily high temperature is 20°C in summer and −3°C in winter (De Jager et al. 2020). Snow and ice cover persist from November through April, and the islands receive ~750 mm of precipitation annually (Risenhoover and Maass 1987). In winter, moose concentrate along shoreline areas where balsam fir (*Abies balsamea*) is ~ 60% of the diet, with the remainder woody browse and arboreal lichens (Risenhoover 1987, Parikh et al. 2017, Tischler et al. 2019). The summer spatial distribution and local density of moose on Isle Royale is unknown, but the spring diet of moose includes newly emergent leaves and the summer diet is largely composed of current leaf growth of deciduous plants and aquatic macrophytes (Ackerman 1987, Tischler et al. 2019).

METHODS

Sample Design

We collected aquatic macrophytes and leaves of terrestrial plant at the east and west ends of the island (hereafter E and W, respectively). The E and W sampling sites were delineated by the boundary of the 1936 and 1948 fires, leaving the central portion of the island unsampled. We collected samples between 13 July and 3 August 2002 when plants were mature, as opposed to emergent or senescent. Samples were collected at ≥ 5 E and W sites from the 6 terrestrial species

composing the principal summer diet of moose on Isle Royale: mountain maple (*Acer spicatum*), sugar maple (*A. saccharum*), mountain-ash (*Sorbus* spp.), paper birch (*Betula papyrifera*), yellow birch (*B. alleghaniensis*), and beaked hazelnut (*Corylus cornuta*) (Ackerman 1987). Sites were separated by >200 m and at each we collected 5 green leaves of each species (including the petiole and excluding twigs) at browse height (0.5–3.0 m) from separate but neighboring stems; samples were pooled for analysis.

Moose commonly forage in aquatic habitats during summer and appear to consume aquatic species in proportion to abundance at Isle Royale (Qvarnemark and Sheldon 2004). Consequently, we opportunistically collected dominant (i.e., most abundant) aquatic macrophyte species (identified to the genus) at 3 E and 3 W sites (lakes) used by moose. At each site, 5 subsamples of each species along the shoreline were collected (where available) and pooled for analysis. Since moose are not known to discriminate among aquatic plant parts (i.e., rhizome, stem, flower), we attempted to collect the entire plant, excluding only large and well-rooted rhizomes. To minimize the collection of benthic sediment, we rinsed samples in lake water to remove loose debris prior to placing in plastic sample bags. Due to the paucity of inland lakes in the W, all 3 W sites were in bays of Lake Superior, versus 1 E site. To reduce the potential effect of sampling in Lake Superior, in 2003 we expanded aquatic plant sampling (6–18 July) to include 5 E and 5 W inland aquatic sites. The W sites included lakes, small ponds, or wetland habitats containing open water where moose were observed feeding or evidence of use was identified (e.g., tracks, fecal pellets). To minimize degradation prior to analyses, we cooled samples until freezing them at −20°C within 12 h of collection.

We collected winter forage samples at 7 E and 7 W sites spaced ≥ 200 m apart between 12 January and 10 February 2003. At each site, we collected 5 twigs (current annual growth) from adjacent stems of individual plants from balsam fir, white cedar (*Thuja occidentalis*), mountain-ash, red-osier dogwood (*Cornus stolonifera*), paper birch, and quaking aspen (*Populus tremuloides*). We clipped twigs at the average diameter for each species eaten by moose in winter (Risenhoover 1987). At each site, arboreal lichens of the genera *Usnea* and *Parmelias* were collected from the branches/bark of standing or newly fallen white spruce (*Picea glauca*) and paper birch. Samples were handled and frozen as with the aquatic macrophytes.

Metrics of Quality and Analysis
Indices of forage quality are based upon either the presence of essential plant nutrients (e.g., water, carbohydrate, fat, protein, vitamins, and minerals) or the absence of indigestible structural carbon (C) compounds and toxins (Crawley 1983). Nitrogen (N) availability is considered the most limiting aspect of herbivore nutrition (Crawley 1983). Since rumen microbes can incorporate both organic and inorganic sources of N into the synthesis of amino acids, crude protein (N × 6.25) is a sufficient metric of digestible protein in ruminants (Schwartz and Renecker 2007). The elemental ratio of C:N is also a useful index of gross forage quality as it provides a measure of the relative investment in C structural compounds (associated with reduced quality) per atom of N (associated with enhanced quality) (Crawley 1983, Sterner and Elser 2002). We report crude protein content (%) and C:N mass ratios of forage types as metrics of overall forage quality.

We oven-dried plant tissue at 60 °C for 48 h to constant mass (or longer as needed for aquatic macrophytes) and ground it to fine powder in a ball mill (Spex CertiPrep Inc., Metuchen, New Jersey, USA). All samples were re-dried overnight and stored in a dessicator until subsamples (C: 1.5 ± 0.1 mg, N: 3.0 ± 0.1 mg) were weighed into tin cups. Subsamples were combusted in a Costech Elemental Combustion System 4010 elemental analyzer (Costech Analytical Technologies, Valencia, California, USA) to measure C and N content. The instrument was calibrated with acetanilide and internal organic check standards were analyzed every 10 samples; analytical precisions were %C ± 0.20 and %N ± 0.05. Duplicate samples were analyzed every 5 samples and results were accepted only if the variance between duplicates was less than that of the standards.

We calculated standard errors of C:N ratios using error propagation, which derives a composite error from that of its component parts (Sterner and Elser 2002). Differences in crude protein and C:N ratios among pooled forage types (terrestrial, aquatic), terrestrial species, sampling location (E and W), and sampling year (2002, 2003) of aquatic macrophytes were tested separately using univariate analysis of variance (ANOVA) (SAS Institute Inc., Cary, North Carolina, USA) due to the unbalanced nature of the data with respect to aquatic sampling location.

We examined crude protein content and C:N ratios among all known moose forage types (i.e., summer and winter) for correlation without *a priori* predictions as to the nature of the relationship. We included data on winter forage types (terrestrial plant twigs and lichens) in this analysis to increase the range of values used to model this correlation. We used the best-fit model describing the correlation for only summer terrestrial leaves as a baseline for comparing the observed and predicted relationship for submergent and emergent aquatic macrophytes using univariate ANOVA. We determined the best fit model by visual assessment and

improvements in R^2. Where ANOVA results were significant ($P < 0.05$), we used Tukey's Honestly Significant Difference (HSD) to determine which samples differed. All tests were considered significant at the $\alpha = 0.05$ level and assumptions of normal distribution and homogeneous variance were tested. Where the assumption of homogeneous variance was violated, individual comparisons were made with two-sample t-tests assuming unequal variance. We report means ± standard errors, unless otherwise noted.

RESULTS

The aquatic macrophyte samples (n = 26; 17 Lake Superior, 9 inland) were from 7 genera in 7 families in 2002, increasing to 88 samples from 27 genera in 15 families in 2003 (Table 1). Samples collected from Lake Superior in 2002 had lower C:N ratios ($\bar{x} = 13.7 \pm 0.7$) than those collected from inland lakes (= 18.0 ± 1.2; $F_{1,24} = 11.07$, $P = 0.0028$); crude protein content did not differ between lake types ($t_{22} = 0.10$; $P = 0.92$). We detected no annual difference in crude protein content of aquatic plants by sample year ($F_{1,86} = 1.01$, $P = 0.3172$); however, the C:N ratio of aquatic samples was higher ($t_{76} = -2.95$, $P = 0.0043$) in 2003 (C:N = 18.3 ± 0.8) than in 2002 (C:N = 15.2 ± 0.7). The C:N ratio distribution was non-normal in the 2003 aquatic samples, in large part due to two outliers that were emergent taxa that contain more structural compounds and expected to have a higher C:N ratio than submerged plants. Removal of the emergent species (n = 15, all collected in 2003) from the analysis resulted in a normally distributed dataset with no difference in crude protein and C:N ratios between years. Therefore, the aquatic macrophyte data we present in comparisons among forage types represent only submergent aquatic data pooled across years, and includes both inland and Lake Superior samples. Alternative analyses using unpooled aquatic

Table 1. Mean crude protein content (%) and carbon:nitrogen (C:N) ratios (±SD) of aquatic macrophyte taxa sampled at Isle Royale National Park during 2002–2003.

Taxon	n	Crude protein	C:N
Emergent			
Juncus	1	17.7	15.4
Sagittaria	1	14.8	14.6
Asteraceae: unk.	1	14.0	15.1
Lysimachia	2	13.7 ± 2.7	18.5 ± 2.1
Eupatorium	1	13.1	17.4
Eliocharus	2	13.0 ± 3.1	14.9 ± 3.4
Menyanthes	1	12.0	24.3
Poaceae: unk.	3	10.4 ± 1.9	25.3 ± 7.9
Equisetum	1	9.6	25.3
Dulichium	1	9.5	28.1
Carex	1	5.8	49
Submerged			
Nuphar	5	22.9 ± 2.7	12.0 ± 1.0
Potamogeton	28	15.3 ± 3.0	16.9 ± 3.4
Brasenia	2	14.9 ± 7.3	17.8 ± 5.1
Elodea	1	14.2	17.0
Myriophyllum	5	14.0 ± 1.7	14.6 ± 2.0
Lemna	1	13.8	18.4
Najas	3	13.7 ± 4.3	13.7 ± 1.6
Sparganium	10	13.4 ± 0.6	19.4 ± 0.9
Utricularia	7	12.1 ± 1.1	15.0 ± 1.8
Megalodonta	1	11.6	17.0
Scirpus	2	11.4 ± 1.2	20.3 ± 1.7
Isoetes	1	11.3	16.5
Vallisneria	1	8.9	21.5
Sclerolepis	1	7.0	11.7
Chara	3	6.8 ± 0.7	17.5 ± 1.8
Ranunculus	1	4.5	15.0

data including all taxa (submergent and emergent) did not alter the statistical significance of the comparison (Supplemental Table).

Submergent aquatic macrophytes had higher crude protein ($T_{110} = 2.9$, $P < 0.0001$; Fig. 1) and lower C:N ratios ($T_{104} = -10.6$, $P = 0.004$; Fig. 1) than terrestrial plant leaves. Since aquatic macrophyte
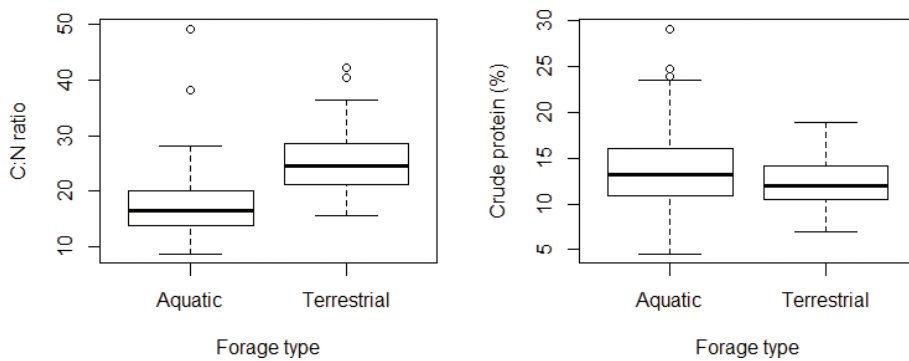
Fig. 1. Boxplot of crude protein content and carbon:nitrogen (C:N) ratios of aquatic and terrestrial moose forage types from Isle Royale National Park, Michigan, USA, 2002. Boxes depict interquartile range, dark lines are median values, circles are outliers, and whiskers are 1.5× interquartile range. Aquatic macrophytes are submergent species pooled across sampling years.

taxa were collected opportunistically, sample sizes were too small to compare forage quality among taxa. Nevertheless, yellow pond lily (*Nuphar*) had the highest mean protein content and a low C:N ratio (Table 1). Among terrestrial forage species, leaves from yellow birch, beaked hazelnut, and paper birch had higher crude protein ($F_{5,54} = 6.86, P < 0.0001$; Fig. 2a) and lower C:N ratio ($F_{5,54} = 8.53, P < 0.0001$; Fig. 2b) than leaves from sugar maple. The trend in C:N ratios across these terrestrial species mirrored that of crude protein content with no exceptions (Figs. 2a, b).

Crude protein content of aquatic macrophytes was higher ($F_{1,86} = 6.56, P = 0.012$) at W sites ($\bar{x} = 15.1\% \pm 0.5$) than E sites ($\bar{x} = 12.6\% \pm 0.9$), whereas the C:N ratio was lower at W sites ($T_{2,24} = -3.3, P = 0.003$). Among summer terrestrial species, only sugar maple differed by location, with crude protein higher ($F_{1,10} = 5.35, P = 0.046$) at W ($\bar{x} = 10.3\% \pm 0.5$) than E sites ($\bar{x} = 8.5\% \pm 0.5$). The C:N ratio of sugar maple at W sites ($\bar{x} = 29.4 \pm 1.7$) was correspondingly lower than at E sites ($\bar{x} = 35.0 \pm 2.2$), but not different ($F_{1,10} = 3.67, P = 0.088$).

The crude protein content and C:N ratios of terrestrial forage types (i.e., summer leaves, winter twigs, and winter lichens) were strongly correlated ($R^2 = 0.97$; Fig. 3a). This relationship was best explained by a negative exponential model: $y = \beta_0 \times e^{-\beta_1 x}$, where x = percent crude protein and y = C:N ratio. Aquatic macrophytes did not fit the exponential model describing summer terrestrial leaves ($y = 69.006 \times e^{-0.0843x}$, $SE_{b1} = 0.228$, $F_{2,145} = 23.7$, $P < 0.0001$), showing instead a high degree of variability in C:N ratios, particularly at low levels of protein (Fig. 3a). Among aquatic macrophytes, the relationship between crude protein content and C:N ratio of emergent taxa followed the terrestrial curve more closely than submergent taxa (Fig. 3b), but was not different (*t*-test, $P = 0.065$). Sample variances among forage types were non-homogenous, although the ANOVA and *t*-test results agreed.

DISCUSSION

Because crude protein content and C:N ratios are indicative of forage quality, our results support the hypothesis that aquatic macrophytes provide high quality summer forage to moose, complementing their consumption of terrestrial plants. On Isle Royale, aquatic macrophytes have ~20% higher crude protein and 40% lower C:N
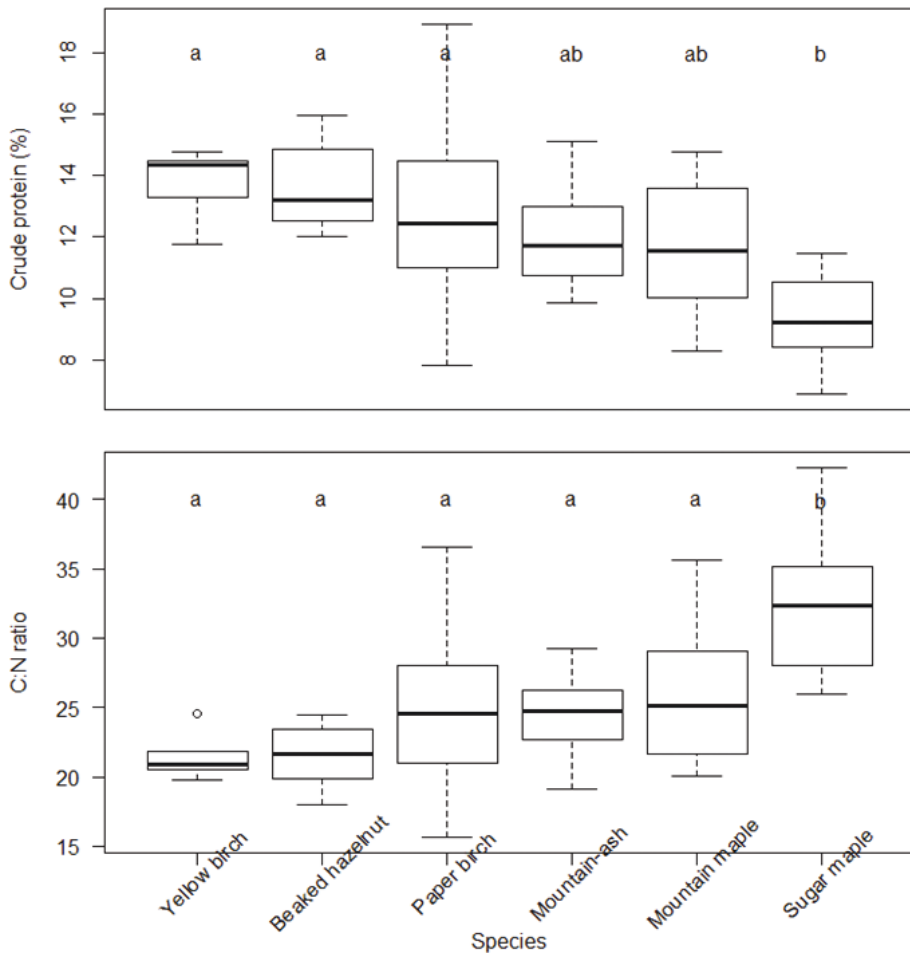
Fig. 2. Boxplot of crude protein content and carbon:nitrogen (C:N) ratios among leaves of summer terrestrial forage species from Isle Royale National Park, Michigan, USA, 2002. Boxes depict interquartile range, dark lines are median values, circles are outliers, and whiskers are 1.5× interquartile range. Letters indicate species that are significantly different.

ratios than terrestrial plant leaves collected in mid-summer. Additionally, the quality of aquatic forage found in bays of Lake Superior appears to be higher than in inland lakes – similar crude protein content and lower C:N ratios. These data support studies on the Copper River Delta in Alaska suggesting that submergent aquatic plants represent an important protein source for moose during summer (MacCracken et al. 1993). In Ontario, moose in a "cafeteria" food trial preferred aquatic species with higher sodium, phosphorus, and crude protein (Fraser et al. 1984). However, crude protein content of terrestrial and aquatic plants did not differ in the Alaskan study, perhaps due to highly variable data, low sample size, and the relatively high protein content (16%) of one terrestrial species, pin cherry (*Prunus pennsylvanica*) (MacCracken et al. 1993). The crude protein content of terrestrial plants in our study was generally similar to levels reported in other studies; however, the aquatic macrophytes had lower crude
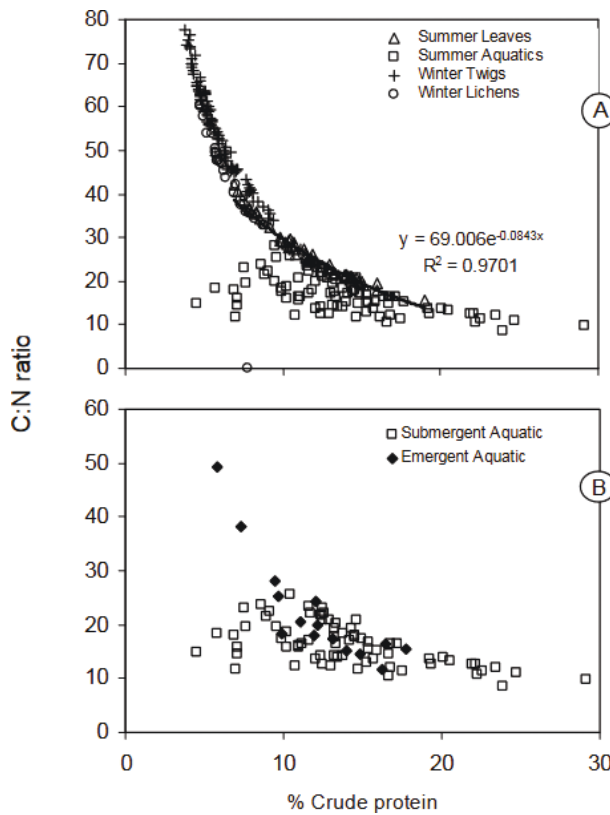
Fig. 3. Correlation between crude protein content (nitrogen [N] × 6.25) and carbon:nitrogen (C:N) ratio among moose forage types (A), and among types of aquatic macrophytes (B) from Isle Royale National Park, Michigan, USA, 2002.

protein than measured in Alaska and Ontario (Table 2; Fraser and Hristienko 1983, MacCracken et al. 1993). Indeed, crude protein content was highly variable within and between lakes on Isle Royale with the minima and maxima ranging 16 and 18%, respectively. The lower crude protein content may reflect differences in species composition, local nutrient inputs at sampling sites, and the sampling period. It is possible that our small sample sizes were not entirely representative of the average crude protein or C:N ratios for certain species (Table 1).

Summer diets of moose in the region of Isle Royale and northeastern Minnesota include measurable amounts of aquatic plants (13–40%) as estimated via stable isotope analysis (Berini 2019, Tischler et al. 2019). Further, moose inhabiting relatively warmer areas of northeastern Minnesota consumed higher amounts of aquatic vegetation (Berini 2019). Consumption of aquatic plants certainly provides highly nutritional forage based on the crude protein and C:N ratios we measured, and moose simultaneously address other nutritional requirements including sodium balance. However, presumably moose diets are necessarily balanced with aquatic and terrestrial vegetation, in part, because aquatic foraging is believed limited by gut fill due to the high water content of aquatic macrophytes and incidental water consumption (Belovsky 1978).

Table 2. Comparison of crude protein content (SD) of summer terrestrial and aquatic forage among studied moose populations.

| Source | Location | % Crude protein | |
		Terrestrial†	Aquatic
This study	Michigan	12 (0.3)	14 (0.6)*
MacCracken et al. (1993)	Alaska	13 (1)#	17 (1)
Fraser et al. (1984)	Ontario	13 (1)#	16 (1)
Crete and Jordan (1982)	Quebec	14 (0.3)‡	na
Renecker and Hudson (1985)	Alberta	13 (0.4)§	na
Oldemeyer et al. (1977)	Alaska	13	na

Note: Samples collected between 30 June and 2 August unless otherwise noted.
†Samples include deciduous leaves and exclude twigs unless otherwise noted.
*Submergent species only.
#Samples include both leaves and twigs.
‡Only beaked hazelnut and mountain maple were sampled; does not represent principal summer diet of moose.
§A composite sampled to reflect diet.

The efficiency with which ingested C is converted into heterotrophic biomass is negatively correlated with forage C:N ratios (Elser et al. 2000). In general, the C:N stoichiometry of freshwater aquatic autotrophs is lower and less variable than that of terrestrial autotrophs up to a magnitude of three between freshwater seston and terrestrial plants (Elser et al. 2000). At Isle Royale, moose feeding on aquatic plants during summer would acquire 1.5 times more N per C atom consumed than acquired through terrestrial foraging during summer, suggesting that the assimilation efficiency of aquatic macrophytes is greater than that of terrestrial plants. This pattern may largely be attributable to physiological constraints that obligate terrestrial plants to a large structural C investment rather than differences in N content per se (Sterner and Elser 2002). Indeed, we found that terrestrial plants had a high and relatively fixed (46–49%) C content while the C content of aquatic macrophytes was highly variable (11–48%), perhaps reflecting that submerged and emergent aquatic macrophytes were pooled for analysis. Furthermore, we found that the C:N stoichiometry of all terrestrial forage types (summer leaves, winter twigs, lichens) followed a tight pattern of exponential decay with increasing crude protein content, whereas aquatic macrophyte C:N ratios were comparatively low and much less predictable across a wide range of crude protein contents. Interestingly, the crude protein-C:N relationship of emergent aquatic macrophytes, which require more structural support than submerged plants, was intermediate that of terrestrial and submergent aquatic plants.

Our results suggest that higher C:N ratios of terrestrial plants are due to greater structural C allocation and lower N content. Thus, even if the difference in crude protein content is not biologically significant, aquatic macrophytes are a higher quality forage than terrestrial plants due to lower concentration of structural C which hinders digestibility. In support, Belovsky and Jordan (1978) reported higher digestibility for aquatic plants (94%) than deciduous leaves (72%) on Isle Royale; albeit, digestibility of both is considered high and deciduous leaves are the principle component of the summer diet of moose. Furthermore, N content was negatively correlated with the

content of phenolics (anti-herbivory compounds) (Jones and Hartley 1999), although the degree to which aquatic macrophytes have evolved chemical defense against herbivory has been little studied (but see Parker et al. 2006).

Pond lilies have historically been identified as an important aquatic forage for moose in North America (Peterson 1955, Cobus 1972). Murie (1934) provided anecdotal evidence of near extirpation of abundant pond lilies in the 1930s by an irrupting moose population on Isle Royale, which may be due to their preference by moose or sensitivity to disturbance (Fraser and Hristienko 1983). More recently, cover of watershield (*Brasenia schreberi*), a previously abundant aquatic macrophyte, has declined in many of Isle Royale's water bodies during periods of high beaver and moose density that coincided with low wolf abundance (Hoy et al. 2019). It is not surprising that we found pond lilies to be a high-quality moose forage (mean crude protein content = 22.9%; Table 1). Among terrestrial species, our results suggest that sugar maple is a low-quality summer moose forage. Sugar maple appears to be an important species in the spring diet of moose on Isle Royale (Ackerman 1987), perhaps due to early leaf emergence and high calcium concentration relative to other terrestrial leaves, but its use declines as forage and diet diversity increase through spring and summer (Belovsky et al. 1973, Krefting 1974, Miquelle and Jordan 1979, Belovsky 1981, Ackerman 1987).

As with northern ungulates, the protein content of the winter diet of moose is insufficient to meet maintenance protein requirements (5–7%, Fig. 3) (Schwartz et al. 1988). Compensation of this "deficit" is achieved principally through catabolizing fat and lean body mass stored during late summer and autumn when forage is up to 3 X more nutritious than in winter (Renecker and Hudson 1986), recycling urea (van Hoven and Boomker 1985), and limiting fetal growth and gestational costs during early-mid winter (Schwartz 2007). Thus, the abundance of high quality forage consumed during summer influences pre-winter body condition and survival (Parker 2003). It follows that consumption of high quality aquatic forage used throughout summer aids post-winter recovery, pre-winter nutritional condition, and winter survival of Isle Royale moose. Surprisingly, in nearby northeastern Minnesota moose in relatively warmer areas consumed poorer diets characterized as high in aquatic forage and low in high-preference terrestrial forage. Further, moose dying overwinter consumed diets higher in aquatic forage than surviving moose (Berini 2019).

The spatial patterns we identified in forage quality among aquatic and terrestrial plant species is consistent with the large-scale spatial (E-W) differences in soil richness and plant species composition on Isle Royale (see Study Area). Spatial heterogeneity in resource quality is widely known to influence browsing behavior and the distribution of herbivores across landscapes (Crawley 1983, Renecker and Hudson 1985, 1986, McNaughton 1988, Fryxell 1991, Wallis DeVries 1996, Parker 2003). Many ungulate populations "track the pulse of production" and "green waves" (Mattson 1980) via seasonal migration, thereby exploiting nutritious forage and maximizing the time period to access such forage when available (Festa-Bianchet 1988, Merkle et al. 2016).

Assuming a similar distribution of macrophytes among aquatic habitats on Isle Royale, ~ 75% of aquatic biomass occurs on the east half of the island based on the length of shoreline available for aquatic foraging. Unfortunately, little is known about or whether moose migration is common on Isle Royale; however, only 2 of 22 radio-collared moose migrated between the east and

west ends of the island in the late 1980s (unpublished data of author, R. O. Peterson). Perhaps this lack of migration can be explained by our observation that while overall forage quality appears to be better on the west end of the island, the biomass of aquatic macrophytes is higher at the east end. Because no large patches of landscape are noticeably devoid of vegetation, except where fire removed it, we recognize that individuals meet their summer-autumn nutritional requirements through a varied diet. Forage quality (protein content), although variable among species in summer, is on a continuum where the majority of terrestrial leaves are nutritious (protein content), highly digestible, and a mainstay of the spring-summer diet across moose range.

The evolution of muzzle anatomy in moose is believed a morphological adaptation for efficient underwater feeding, a behavior unique to moose among cervids (Hofmann 1989, Geist 1998, Clifford and Witmer 2004). Aquatic feeding is undoubtedly an important source of sodium for many moose populations (Botkin et al. 1973, Jordan et al. 1973, Fraser et al. 1984); however, moose on Isle Royale (and elsewhere) can meet sodium requirements at mineral licks which contain much higher sodium concentrations (on a wet-weight basis) than aquatic plants (Risenhoover and Peterson 1986). We propose that aquatic foraging by moose at Isle Royale is also a mechanism to exploit relatively N-rich microsites (aquatic habitats) in an otherwise N-limited landscape (White 2012). Regardless, aquatic habitats provide moose summer forage high in digestible protein critical to physical recovery and growth, while reducing their post-winter sodium deficit, insect harassment, risk of predation, and thermal stress (Morris 2014).

Given the patchy spatial distribution of aquatic habitats on Isle Royale, the positive influence of aquatic feeding on the pre-winter nutritional condition of moose could affect spatial dynamics of winter population density, mortality, and predation rate. We suggest that increased consumption of high quality aquatic macrophytes on the east end of Isle Royale might supplement the lower quality winter forage, thereby foregoing the need to migrate. From this perspective, it is understandable that aquatic foraging by moose is prevalent (Tischler et al. 2019); however, it is unknown if the pre-winter condition of moose differs at the island ends or the time associated with developing a migratory strategy. We encourage further research to test such assumptions and to better understand the relative use and role of aquatic plants on moose population dynamics.

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SUPPLEMENTAL TABLE

Comparison of ANOVA test results for the effect of forage type on plant crude protein content or C:N ratio when aquatic macrophytes are either pooled or separated by sample year (2002, 2003) and type (submergent and emergent, submergent only).

Aquatic dataset	n†	Crude protein		C:N ratio	
		F	*P*	*F*	*P*
2002	26	149.2	<0.0001	229.0	<0.0001
2003	62	118.1	<0.0001	289.8	<0.0001
2002, 2003 combined	88	122.4	<0.0001	367.9	<0.0001
2002, 2003 combined; submergent only	73	124.6	<0.0001	373.7	<0.0001

†Sample size of aquatic macrophyte dataset used for analysis.