Do bulk nitrogen isotope ratios capture declines in captive muskox body condition?

Molly Cook, Garrett Savory, John Blake, Diane O'Brien

Researchers are often interested in studying body condition in wild populations, but this is a difficult task due to logistic issues. We were interested in looking at blood serum stable nitrogen isotope ratios from a sample size of 21 muskox at the Large Animal Research Station at the University of Alaska Fairbanks from 2007-2016. More specifically, we were interested in examining how these values varied during a mortality event from 2009-2010 where many individuals experienced body mass loss. We were also interested in seeing how these nitrogen isotope ratios vary with season, sex, and age.

Hypotheses

We expected to see an increase in $\delta^{15}N$ with a decrease in the mass of the muskox. We expected to see this because between ^{15}N and ^{14}N, ^{14}N is preferentially excreted in the urine as a result of fractionation associated to transamination. If the lighter nitrogen isotope is being excreted and not sufficiently replenished by food, we would expect to see higher levels of ^{15}N being retained in the animal (Macko et al. 1986)*.

In terms of season, we expected to see $\delta^{15}N$ in late winter to be greater than $\delta^{15}N$ in autumn because muskox in the wild consume less food during late winter due to food scarcity, and these habits are often observed in captivity, although food quantity may not vary. We expected $\delta^{15}N$ to be low in autumn, because autumn comes after a season where there is usually ample amounts of food, including pasture grass, which is not as readily available or nutritious after spring and summer. We expected $\delta^{15}N$ in spring and summer to be about equal to early winter, with these values being greater than autumn and less than late winter. The seasons were defined as follows: early winter: October-January, late winter: February-April, spring and summer: May-July, and autumn: August-September (Barboza et al. 2015)*.

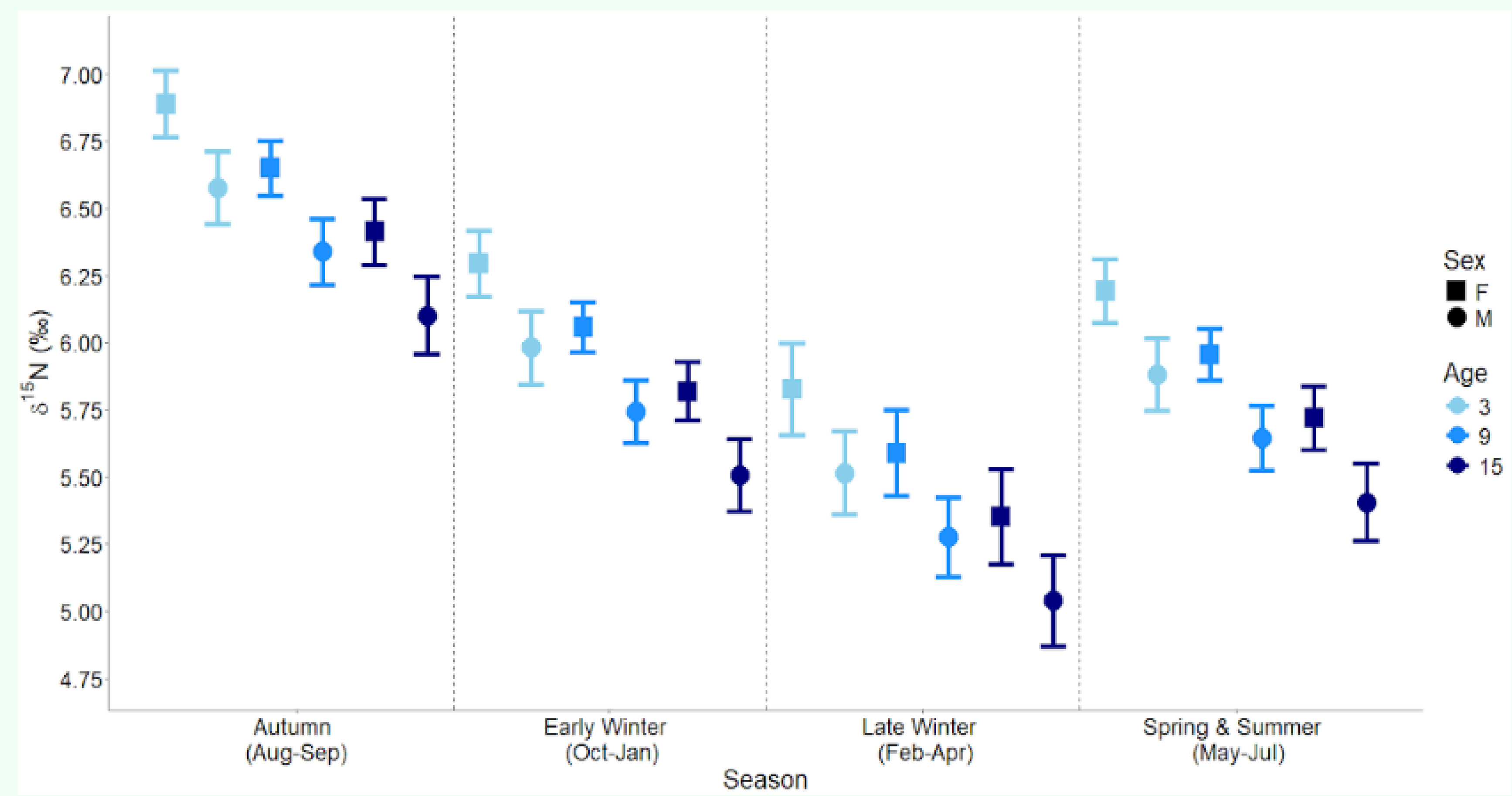
In examining $\delta^{15}N$ values in males versus females, we expected to see larger $\delta^{15}N$ values in males because they are larger than the females, therefore they require more food to maintain body mass.

When it came to examining $\delta^{15}N$ values compared to age, we expected to see higher $\delta^{15}N$ values in older individuals since these individuals are often more sedentary and may eat less due to decreased caloric requirements. Specifically, we expected to see the highest $\delta^{15}N$ values in animals 15 years of age followed by 9 and then 3 years.

Methods

- Blood serum (n = 163) was dried and brought to the Alaska Stable Isotope Facility at the University of Alaska Fairbanks to obtain a nitrogen stable isotope value using an elemental analyzer interfaced with a stable isotope mass spectrometer. Delta notation as per mil (‰) abundance was used to express isotope ratios. (DeltaX = [(rsample/rstandard) - 1] x 1000, where X = $\delta^{15}N$ and rsample and rstandard are the ratios of heavy to light isotopes in the sample with the standard being atmospheric nitrogen. Measurement precision < 0.15%.
- We used a multiple linear regression to test for significance between $\delta^{15}N$ and body mass (kg), season, sex, and age (years). We used an alpha value of 0.05 to assess statistical significance.

Results



Coefficients	Estimate	SE	t-value	p-value
Intercept	7.399	0.294	25.153	< 0.001
Season: Early Winter	-0.594	0.127	-4.672	< 0.001
Season: Late Winter	-1.062	0.174	-6.108	< 0.001
Season: Spring & Summer	-0.695	0.130	-5.348	< 0.001
Age	-0.040	0.012	-3.442	< 0.001
Sex: Male	-0.312	0.115	-2.718	0.007
Body Mass (kg)	-0.002	0.001	-1.422	0.157

p-value: < 0.001, adjusted r^2 value: 0.305, F(6,155): 12.77

We found that when it comes to seasons, $\delta^{15}N$ is high in autumn and low in late winter. When it came to body mass, we saw that there was no significant relationship between body mass and $\delta^{15}N$ values. We found that $\delta^{15}N$ values in females were higher than $\delta^{15}N$ values in males and found that $\delta^{15}N$ values were highest in muskox 3 years of age, followed by 9 and 15 years. The error bars are standard error.

*Barboza, P. S., Peltier, T. C., and Forster, R. J. 2015. Ruminal Fermentation and Fill Change with Season in an Arctic Grazer: Response to Hyperphagia and Hypophagia in Muskoxen.
*Macko, S.A., Fogel Estep, M.L., Engel, M.H., Hare, P.E. 1986. Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. Geochimica et Cosmochimica Acta . 50:2143-2146.

Discussion

We saw that $\delta^{15}N$ was high in autumn and low in early winter. One reason for the $\delta^{15}N$ being higher in autumn may be because intact males do not eat as much during this time of year, which is mating season. One reason $\delta^{15}N$ was low in late winter may be because muskox tend to be more stationary during this time of year as a means to minimize energy expenditure to conserve energy. Additionally, although the quantity of food provided for the muskox does not change during this time of year, food intake decreases during this time of the year, which is what is seen in the wild due to food scarcity during late winter. We must also take individual variation into consideration in terms of metabolism and eating habits.

We saw that the females have higher $\delta^{15}N$ values than males. One reason for this may be because the females have a higher nutrient demand in terms of reproduction. We must also take into account that males and females may use nitrogen isotopes differently and have different metabolisms.

In regards to age, we saw that $\delta^{15}N$ ratios were highest in muskox 3 years of age, followed by 9 and 15 years. Our results were the exact opposite of what we expected. One reason $\delta^{15}N$ ratios may be lowest in older muskox may be because these animals are often more sedentary, which requires less food intake to maintain body mass, so these animals are able to maintain body mass more easily than a more active, younger muskox.

We also saw that there was no significant relationship between $\delta^{15}N$ and mass. Using the difference between expected body mass and actual body mass may be a better variable to use and may have given us a different result.

Since there was not a detailed record available for muskox diet, we were not able to accurately compare $\delta^{15}N$ to that of the diet. This limits our ability to reason that certain variations in $\delta^{15}N$ may have been a partial result of dietary $\delta^{15}N$ changes. It would be interesting to perform a study where we do have access to a more detailed record of diet to see how serum $\delta^{15}N$ ratios may vary with dietary $\delta^{15}N$, and how this may alter our results.

Acknowledgements

I would like to say thank you to the Office of Undergraduate Research and Scholarly Activity at the University of Alaska Fairbanks for providing the necessary funds to proceed with this project via a mentor award. I would also like to thank Pat Rivera, Diana O'Brien, John Blake, and Jessica Johnson for their input on the project. I would especially like to thank Garrett Savory, who has been my mentor throughout the research process.
Research reported in this publication was additionally supported by the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103395. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the NIH