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## Variation in Muskox (*Ovibos moschatus*) Guard Hair Growth Rates: Implications for Measuring Chronological Biomarkers

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ABSTRACT: Segmental analyses of hair may be useful for measuring biomarkers over several seasons to years from a single sample. To attribute hair segments to specific time periods, a known chronological marker, or a hair growth rate, is needed. We examined guard hair growth rates of captive muskoxen (Ovibos moschatus) in Fairbanks, Alaska, USA. We sought to determine if a general growth rate could be applied across muskox populations, thus facilitating the use of segmental analyses for various biomarkers. We used archived samples from 16 muskoxen that had guard hairs sampled at six, 14, and 30 wk after shaving. We measured the lengths of 10 guard hairs per sample, calculated weekly and annual growth rates, and then fitted linear mixedeffects models to assess the effect of different covariates on hair growth rate. The period in which hair had been grown had a significant effect (P < 0.05) on growth rate. Extrapolated annual hair growth rates were 277±40 mm/yr (weeks 0-6), 248±47 mm/yr (weeks 7-14), and  $165\pm36$  mm/yr (weeks 15-30), with an overall average rate of 210±14 mm/yr. These rates were significantly faster than those of freeranging Greenland muskoxen-78 mm/yr as measured by stable isotope analyses-and varied intra-annually. This suggests that a universal growth rate cannot be generalized across muskox populations and time.

*Key words:* Biomarkers, guard hair, hair growth rate, muskox, *Ovibos moschatus*, segmental analysis.

Biomarkers are measurable characteristics or substances that indicate biological processes or physiological states within an animal (Califf 2018). In wildlife, biomarkers can be used as an indicator of individual and population health (Downs et al. 2018). Biomarkers that can be measured from samples that are collected noninvasively and opportunistically, such as during capture or harvest, are desirable.

In the Canadian Arctic, biomarkers of health in muskoxen (*Ovibos moschatus*) and

caribou (*Rangifer tarandus*), important cultural and subsistence species for the Inuit, are being monitored through an ongoing community-based wildlife health program (Carlsson et al. 2016). Harvesters submit biological samples, including hair, from animals that they harvest for food. This program provides valuable information on the health status of these species through the measurement of various physiological, nutritional, and infectious disease biomarkers.

Hair is a useful matrix for tracking biomarkers, and may be a preferred sample type, as it can be collected noninvasively and easily by laypeople. Hair receives substances from its vascular supply that are incorporated into the hair shaft during (Macbeth et al. 2010; Di Francesco et al. 2021) or after (Cattet et al. 2014) growth. Segmental analyses of hair, where hair is sectioned in segments corresponding to different time periods (seasons or years), have been used to document the historical chronology of a biomarker from a single sampling event (Carlitz et al. 2014). Such biomarkers may indicate prior events or health states that inform about past or future fitness (Downs et al. 2018). To relate growth time frames to specific segments of hair, a standardized chronological marker (i.e., a measurable parameter known to occur at a given time) or a known hair growth rate is required. Segmental analyses consequently become more practical if a species has a common or generalizable hair growth rate across populations or regions. Several studies using segmental analyses of hair in people and domestic animals have assumed a standard hair growth rate, whereas others have recognized variability of hair growth rates among individual people and horses (Equus ferus caballus; LeBeau et al. 2011; Burnik Šturm et al. 2015; Duran et al. 2017).

Muskoxen grow their woolly undercoat, qiviut, from April to November, and shed it entirely the following spring. Measurement of biomarkers, such as cortisol, in the qiviut has revealed spatial, sex, and annual variations (Di Francesco et al. 2017). Muskoxen also have guard hairs, which are grown over multiple years and are shed and replaced continuously (Flood et al. 1989). Through segmental analyses, these guard hairs may have the potential to provide longer-term measures of biomarkers in muskoxen (Mosbacher et al. 2016).

With the goal of maximizing the information that can be derived from samples submitted through community- and capture-based wildlife health monitoring programs, we sought to determine if muskox guard hairs could be used to provide a historic, multiyear chronology of biomarkers. In a free-ranging muskox population in northeast Greenland, the guard hair growth rate has been estimated at 78 mm/yr on the basis of seasonal stable isotope signatures (Mosbacher et al. 2016). Our aim was to explore the prospect of a generalizable guard hair growth rate in muskoxen, as it would allow for standardization for segmental analyses and measurement of biomarkers.

We accessed archived samples of guard hairs collected from a captive muskox population at the University of Alaska Fairbanks, Fairbanks, Alaska, USA as part of an experiment on qiviut cortisol (Di Francesco et al. 2021). The original study included 16 muskoxen that were sampled to assess the effects of adrenocorticotropic hormone (ACTH) administration on giviut cortisol concentrations. Methods are described fully in Di Francesco et al. (2021). Briefly, muskoxen had patches of hair shaved from the rump, shoulder, and neck on 23 or 24 July 2018 and were administered weekly intramuscular injections of physiological saline (n=6, control group) or synthetic ACTH (n=10, treatment group) for 5 wk. Hair samples, representing 6 wk (4-5 September 2018), 14 wk (31 October to 2 November 2018), and 30 wk (19–21 February 2019) of growth, were subsequently shaved and collected from the patch on the rump. Hair was also shaved and collected from the patches on the shoulder and neck at 6 wk, but not at subsequent collection periods. Samples were stored dry at room temperature in paper envelopes until examined.

The lengths of guard hairs were measured from each body region, animal, and collection period. Hairs were placed in common orientation and the longest 10 of approximately 200– 400 collected guard hairs were selected, as they were most likely to have been growing for the longest time during that period. Hair lengths were measured to the nearest millimeter using a standard ruler with minimum graduation of 1 mm.

The mean weekly and extrapolated annual growth rates were calculated for each period and body location. Separate linear mixedeffects models, with animal identity as the random effect, were used to assess differences in weekly guard hair growth rates among periods of growth and body locations, respectively. The potential effects of ACTH treatment, sex, and age, as well as their interactions with period of growth or body location, on guard hair growth rate were also investigated. All variables were first assessed using univariate models and only those with P < 0.2 were kept for multivariate model selection. The Akaike information criterion corrected for small sample size (AICc; Bedrick and Tsai 1994) was used for model selection, where the model candidates with the lowest AICc were chosen as the best-fit models (Supplementary Material Tables S1 and S2). Model assumptions were assessed by reviewing residual plots. All statistical analyses were performed using R software (R Core Team 2022) and the lme4 (Bates et al. 2015) and MuMIn (Bartoń 2023) packages. The significance level was set at P < 0.05.

When assessing the effect of period of growth on rump guard hair growth rate, the final model included only period of growth (Table S3). When assessing the effect of body location on guard hair growth rate, the final model included only body location (Table S4).



FIGURE 1. Growth rates (mm/wk) of muskox (*Ovibos moschatus*) guard hairs collected from the rump and grown during weeks 0–6, 7–14, and 15–30 after the initial shaving. Black triangles correspond to the means and black circles to the outliers. Significant differences between groups are indicated by the horizontal bars. \*\*\* corresponds to  $P \le 0.0001$ . Samples were collected from 16 captive muskoxen at the R. G. White Large Animal Research Station of the University of Alaska Fairbanks, Fairbanks, Alaska, USA from July 2018 to February 2019 as part of a study by Di Francesco et al. (2021).

Age, sex, ACTH treatment, and interaction terms were not included in the final models.

Hair growth rates were significantly faster during weeks 0–6 (P<0.0001) and 7– 15 (P<0.0001) compared with weeks 15–30, but growth rate did not vary significantly (P=0.052) between weeks 0–6 and 7–15 (Fig. 1, Table 1). Hair growth rates were significantly faster on the neck compared with the shoulder (P<0.0001) and the rump (P<0.0001), but shoulder and rump rates did not differ significantly (P=0.67; Fig. 2, Table 2).

The guard hair growth rate that we determined in these captive muskoxen differed between body regions, varied intra-annually, and was considerably faster than the 78 mm/  $\,$ yr growth rate reported in free-ranging muskoxen in Greenland (Mosbacher et al. 2016). Our findings show that the application of a generalizable guard hair growth rate across muskox populations is not possible. The variability of hair growth rates that we observed may be attributable to a variety of factors, including season, climate, nutrition, study methods, and demographics of the animals sampled, and these variables should be considered in future studies using hair for measurement of biomarkers.

We found that guard hair growth was faster on the neck compared with the shoulder and the rump. Differences in hair growth rates across body locations have also been demonstrated in domestic dogs (*Canis familiaris*; Gunaratnam and Wilkinson 1983) and cattle (*Bos taurus*; Burnett et al. 2014). Similarly, variations in hair biomarker concentrations at different body locations have been noted in both caribou and muskoxen (Di Francesco et al. 2021; Rakic et al. 2023). These differences emphasize the importance of standardizing body location for sampling.

We also observed variability in guard hair growth rate over time. Growth rate was significantly slower in weeks 15–30 (November to February) compared with the earlier periods.

TABLE 1. Mean length and growth rates of muskox (*Ovibos moschatus*) guard hairs collected from the rump and grown during different periods after the initial shaving. Samples were collected from captive muskoxen at the R. G. White Large Animal Research Station of the University of Alaska Fairbanks, Fairbanks, Alaska, USA from July 2018 to February 2019 as part of a study by Di Francesco et al. (2021). Data are presented as mean  $\pm$  SD (n=16).

Period grown	Hair length (mm)	Weekly growth rate (mm/wk)	Extrapolated annual growth rate (mm/yr)
Weeks 0–6	33±5	$5.3 \pm 0.8$	$277 \pm 40$
Weeks 7-14	$39 \pm 7$	$4.7 \pm 0.9$	$248 \pm 47$
Weeks 15–30	$50 \pm 11$	$3.2 \pm 0.7$	$165 \pm 36$
Weeks 0-14	$71 \pm 7$	$5.0 \pm 0.5$	$260 \pm 27$
Weeks 0-30	$121 \pm 8$	$4.0 \pm 0.3$	$210 \pm 14$



FIGURE 2. Growth rates (mm/wk) of muskox (*Ovibos moschatus*) guard hairs collected from the rump, shoulder, and neck and grown during weeks 0–6 after shaving. Black triangles correspond to the means and black circles to the outliers. Significant differences between groups are indicated by the horizontal bars. \*\*\* corresponds to  $P \le 0.0001$ . Samples were collected from 16 captive muskoxen at the R. G. White Large Animal Research Station of the University of Alaska Fairbanks, Fairbanks, Alaska, USA from July 2018 to February 2019 as part of a study by Di Francesco et al. (2021).

It is probable that this is a seasonal photoperiod effect, and is consistent with observations by Flood et al. (1989) of fewer active guard hair follicles on the rump in captive muskoxen between November and March. Slower hair growth rates in the winter have also been reported in domestic sheep (*Ovis aries*) and goats (*Capra hircus*; Sumner and Bigham 1993). Diet quantity and quality may contribute to intra-annual variations in hair growth rate, as seen with qiviut (Robertson 2000); however, in both our study and that of Flood et al. (1989), the captive muskoxen were on relatively consistent high-quality diets throughout the year, so this is unlikely to have been a factor in the intra-annual variability that we saw. Finally, shaving hair may affect hair growth rates, but studies have reported conflicting results as to whether there is an effect and whether it is positive or negative (Bigham 1974; Gunaratnam and Wilkinson 1983). A study on muskox qiviut growth found that no compensatory qiviut growth occurred after clipping (Robertson 2000).

Our results differed substantially from those of Mosbacher et al. (2016), with the guard hair growth rate in our study two to three times faster than that observed in wild Greenland muskoxen. Several factors may have contributed to this difference. Whereas the captive muskoxen were on a high and constant nutritional plane (Di Francesco et al. 2021), forage quality and quantity of the freeranging population in northeast Greenland vary considerably within and among years (Mosbacher et al. 2016). Although these authors did not describe intra-annual variation in hair growth rates, it may be that their methods would not have detected such an effect. Climate and spatial factors may also influence hair growth rates. For example, our study location, Fairbanks, Alaska (64° 52'50.2"N, 147°52'06.2"W), has a continental-cool boreal bioclimate with a mean annual temperature of -2 C (Jorgensen and Meidinger 2015), whereas the study in Greenland (74°28'N, 20°34'W) was in the high Arctic, where the mean annual temperature is -9 C (Mosbacher et al. 2016).

TABLE 2. Mean length and growth rates of muskox (*Ovibos moschatus*) guard hairs collected from the rump, shoulder, and neck and grown during weeks 0–6 after shaving. Samples were collected from captive muskoxen at the R. G. White Large Animal Research Station of the University of Alaska Fairbanks, Fairbanks, Alaska, USA from July 2018 to February 2019 as part of a study by Di Francesco et al. (2021). Data are presented as mean  $\pm$  SD (n=16).

Body location grown	Hair length (mm)	Weekly growth rate (mm/wk)	Extrapolated annual growth rate (mm/yr)
Rump	33±5	$5.3 \pm 0.8$	$277 \pm 40$
Shoulder	33±3	$5.4 \pm 0.5$	$281 \pm 27$
Neck	38±3	$6.1 \pm 0.6$	$321 \pm 29$

The methods used to determine hair growth rates may also have contributed to the differences between studies. We used a direct measurement of the length of the longest guard hairs grown during specific periods after shaving. Recognizing that we were selecting from a mixed-hair population that probably began growing at different times, our intent was to choose those that had been growing throughout the entire period (Flood et al. 1989). This selection process may have resulted in our underestimating the true variability in an individual's hair growth rates. In contrast, Mosbacher et al. (2016) inferred annual growth rate on the basis of patterns of nitrogen stable isotopes. In that study, guard hairs clipped from 10 chemically immobilized adult muskoxen in October 2013 were cut in 2-mm sections and analyzed for nitrogen stable isotopes sequentially. Periodicity in dietary chronology, as inferred by the stable isotopes, was converted to an annual hair growth rate (Mosbacher et al. 2016).

In both studies, the small sample sizes and demographics of animals sampled may have contributed to the differences in growth rates. Our study included males and females, ranging in age from 1 to 11 yr (Di Francesco et al. 2021), whereas Mosbacher et al. (2016) focused on adult females. Although age and sex may influence giviut fiber yield (Robertson 2000; Rowell et al. 2001), we did not detect a significant effect of sex or age on guard hair growth rate. We also did not detect a significant effect of ACTH treatment on guard hair growth rate. Nevertheless, demographic and treatment differences are important to consider for comparisons between populations and individuals.

Our results do not support a guard hair growth rate that can be generalized across muskox populations. The nearly threefold higher growth rate that we report compared with that for wild Greenland muskoxen probably reflects two extremes of the growth spectrum: wild muskoxen living in a climatically severe and nutritionally limited region versus captive muskoxen living in a relatively mild climatic region and on a high nutritional plain. Across their range, muskoxen are exposed to considerable environmental, climatic, and nutritional differences, as well as substantial interannual variability in determinants of health. All these elements will affect hair growth rates, even within a population. It follows that the use of a single guard hair growth rate for retrospective chronological analysis of hair biomarkers is not applicable across muskox populations; intra-annual variability adds further complexity to this story. Rather, hair growth patterns at a regional, population, or even subpopulation level, and on an annual basis, need consideration. Although it would be very attractive to use segmental analyses for a long-term measure of biomarkers, our work has highlighted the natural variability of hair growth rate in muskoxen. More broadly, our study emphasizes the importance of considering hair growth dynamics in any wildlife species and that hair growth rates should be inferred from standard chronological biomarkers for more accurate segmentation, rather than applying a generalized growth rate.

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## SUPPLEMENTARY MATERIAL

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