Muskox Aging Protocol

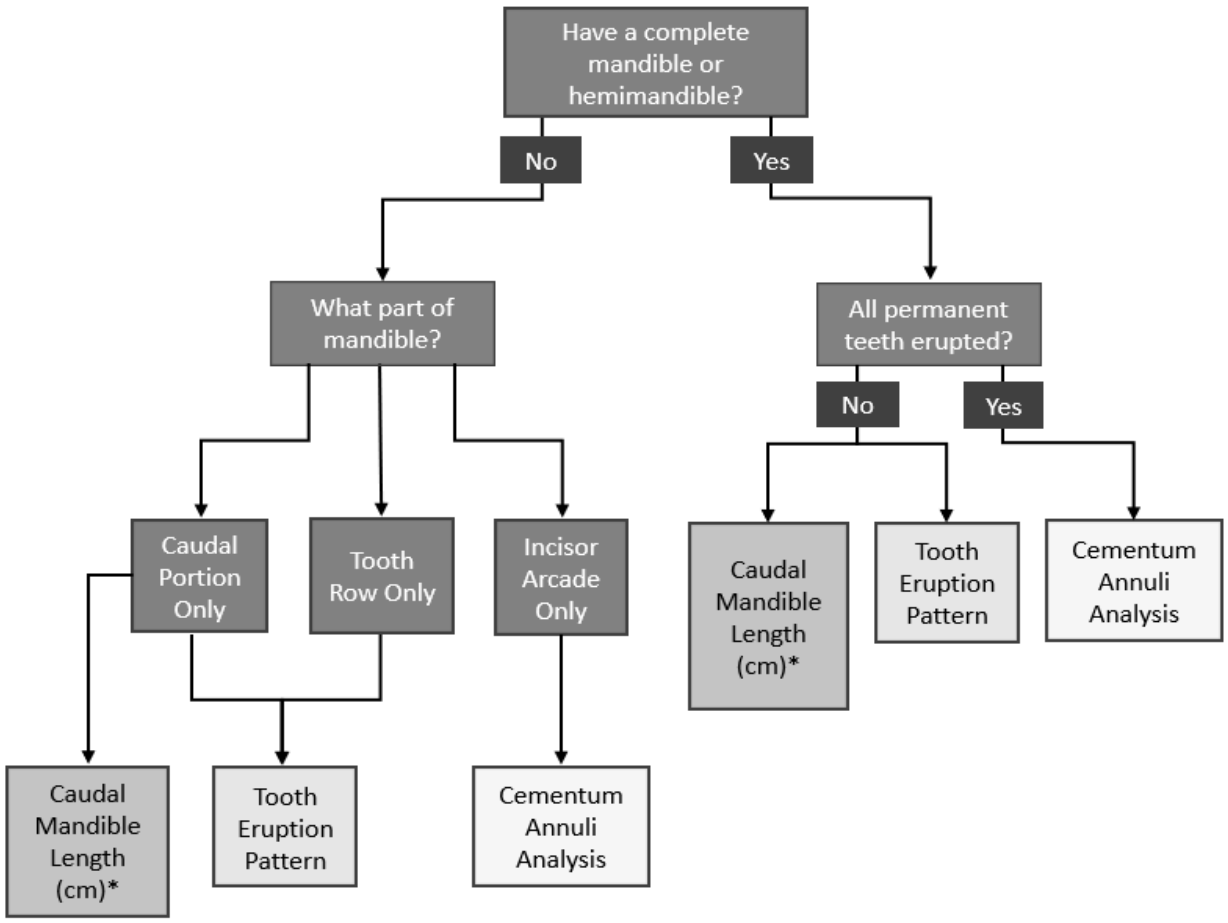
Materials

- Large calipers
- Scalpel blade and handle
- Forceps
- Necropsy knife
- Small and large whirl-paks
- Dental elevator and extraction forceps
- Coin envelopes
- Camera

Before you start work

The mandible bones should be stored at -20°C until time of processing if mandibles are fresh. The jaw bones should be removed from the freezer and left in a cool room to thaw overnight. If dried then freezer storage is not necessary.

Overview of sample available and aging method



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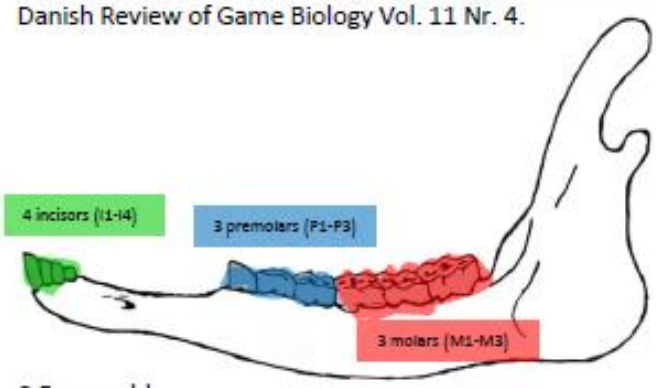
Muskox Age Estimation

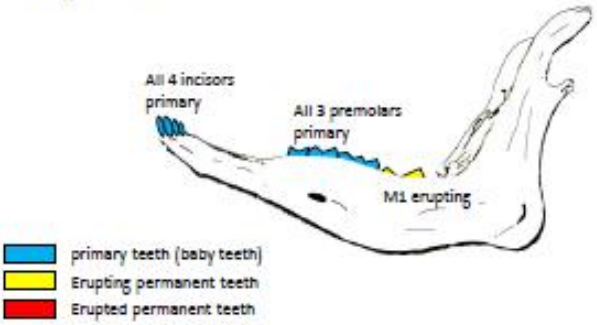
Adapted from Henrichsen and Grue, 1980, *Age Criteria in the Muskox (*Ovibos moschatus*) from Greenland*, Danish Review of Game Biology Vol. 11 Nr. 4.



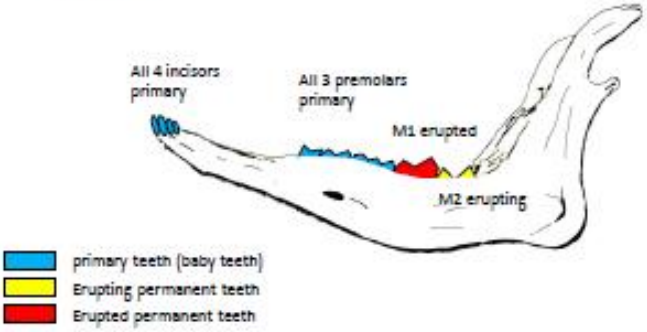
Please note:

- Incisor 4 (I4) is sometimes missing.
- primary premolars have one (P1), two (P2) and three (P3) points. Permanent premolars have only one point each.
- Teeth eruption can vary between animals and may not correspond exactly to the guidelines presented here.

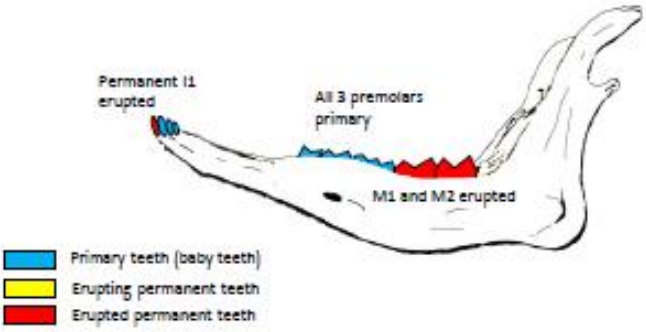
0.5 year old



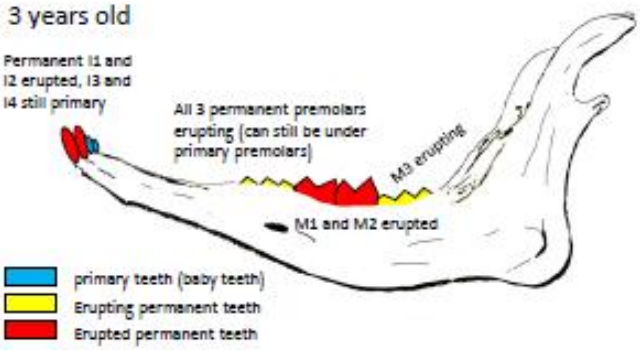
1 years old



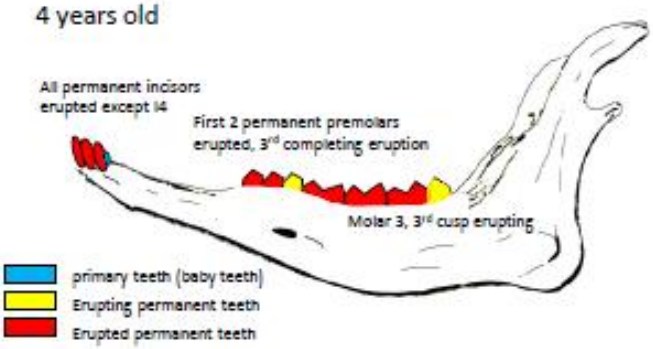
2 years old



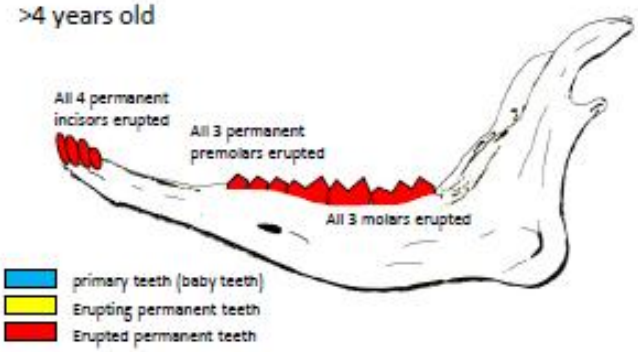
3 years old



4 years old



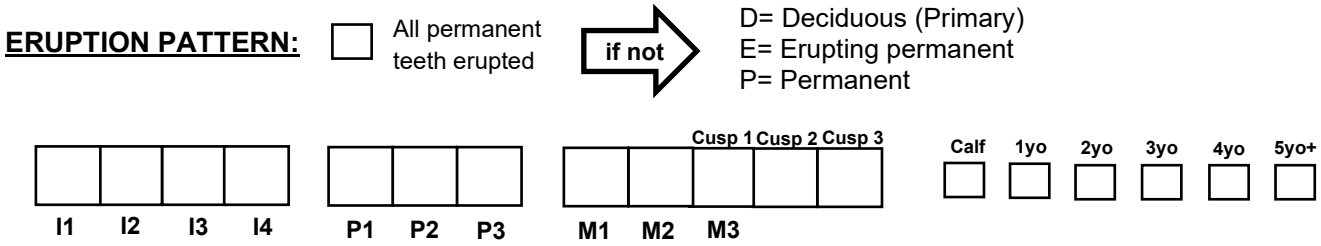
>4 years old



Erica Suitor and Fabien Mavrot, Kutz Lab, University of Calgary

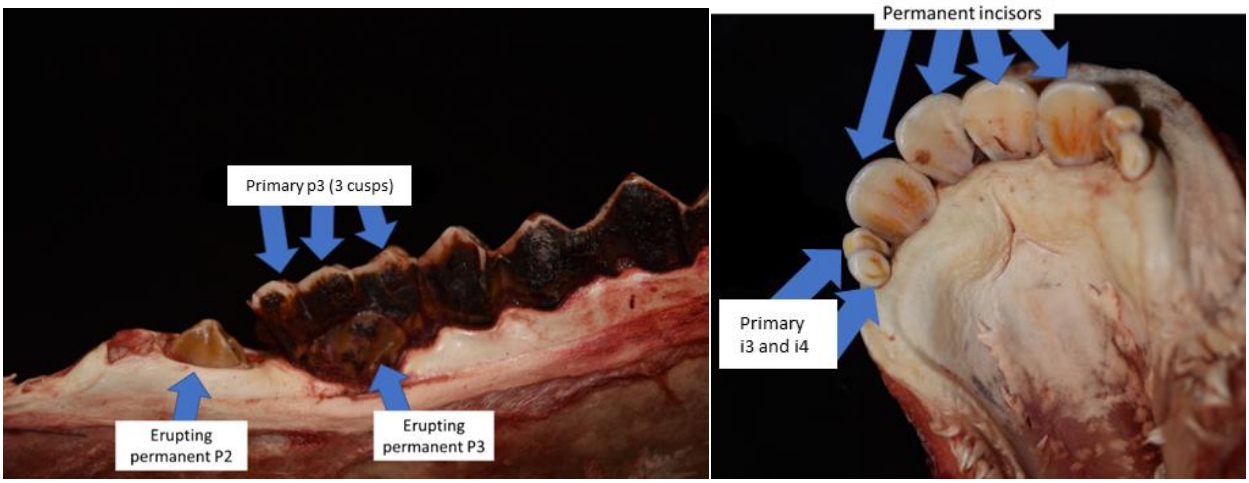
Age determination based on teeth eruption in muskoxen

Muskoxen can be aged by tooth eruption up to 5 years old and can only be aged by month if month of death and an estimated birth date of May 1st is used. Eruption pattern can be recorded per tooth which can then correspond of their age.

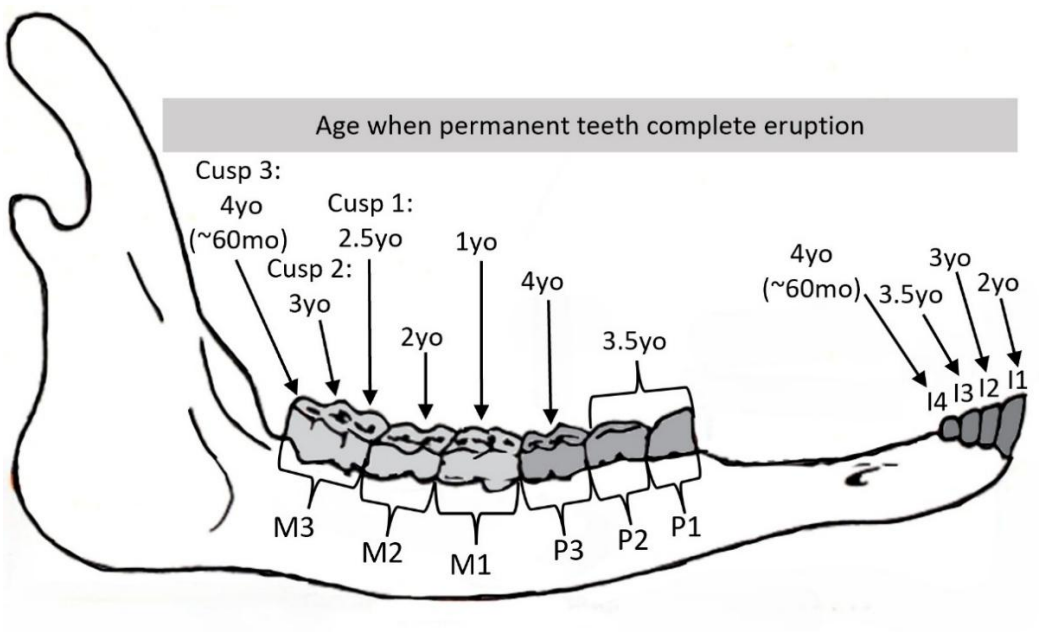


Please note:

- Teeth eruption can vary between animals and may not correspond exactly to the guidelines presented here.
- Incisor 4 (I4) is sometimes missing or can erupt later than 4 years old; therefore, they are not always reliable to age.
- Primary premolars have one (p1), two (p2) and three (p3) cusps (points). Permanent premolars have only one cusp each.
- During their fourth year the premolar precursors may be present or not, it depends on the handling of the sample. It is important to look at the eruption of molar 3 to determine age.
- Molar 3 erupts for the longest period of time and is very important for determining age. If the M3 cusp 3 is still erupting this animal is around 4 years old. Once all the teeth are erupted it can be classified as 5+ years old.



Primary and permanent teeth in a muskox.



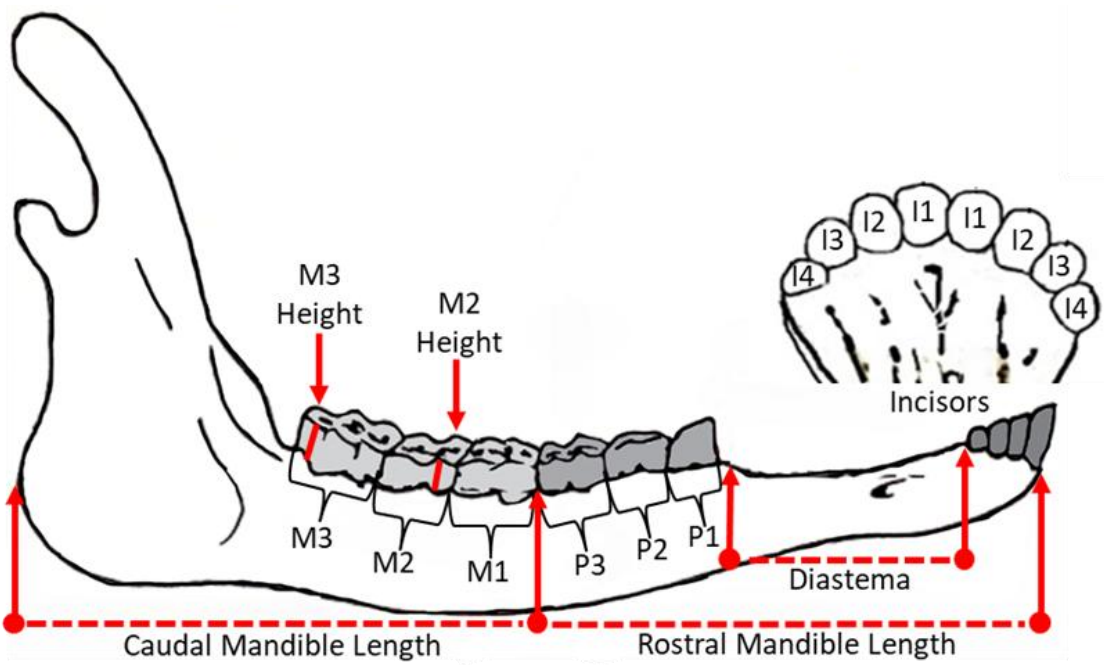
Aging with Mandible Morphometrics

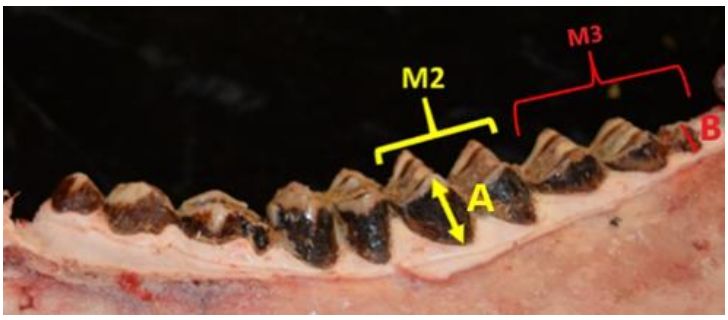
To test the utility of mandible morphometrics for aging muskoxen, We examined 178 archived hemimandibles with known sex from Banks Island, NT. The uniform cleaned and dried state of these mandibles allowed reliable morphometric measurements from a single muskox population.

1. Each mandible was aged based on tooth eruption patterns as we used the tooth eruption age to compare to the morphometrics.
2. The diastema, rostral, and caudal mandible lengths were measured with calipers. Rostral and caudal mandible lengths were summed to calculate the total mandible length. **A ruler was used to measure height of molar 2 (M2) and M3, from the cervical junction between the root and crown to the crown tip.**

Description of measurements:

Morphometric	***Description***
Diastema (cm)	Measurement of the gap caudal point I_4 and rostral of P_1
Rostral Mandible Length (cm)	From the most rostral point of the mandible to caudal point of P_3
Caudal Mandible Length (cm)	Most caudal point of P_3 to the most caudal part of the mandible
Molar 2 Height (mm)	Measurement from the gumline to the tip of the crown of the 1st cusp
Molar 3 Height (mm)	Measurement from the gumline to the tip of the crown of the 3rd cusp
Total Mandible Length (cm)	Addition of rostral and caudal mandible length

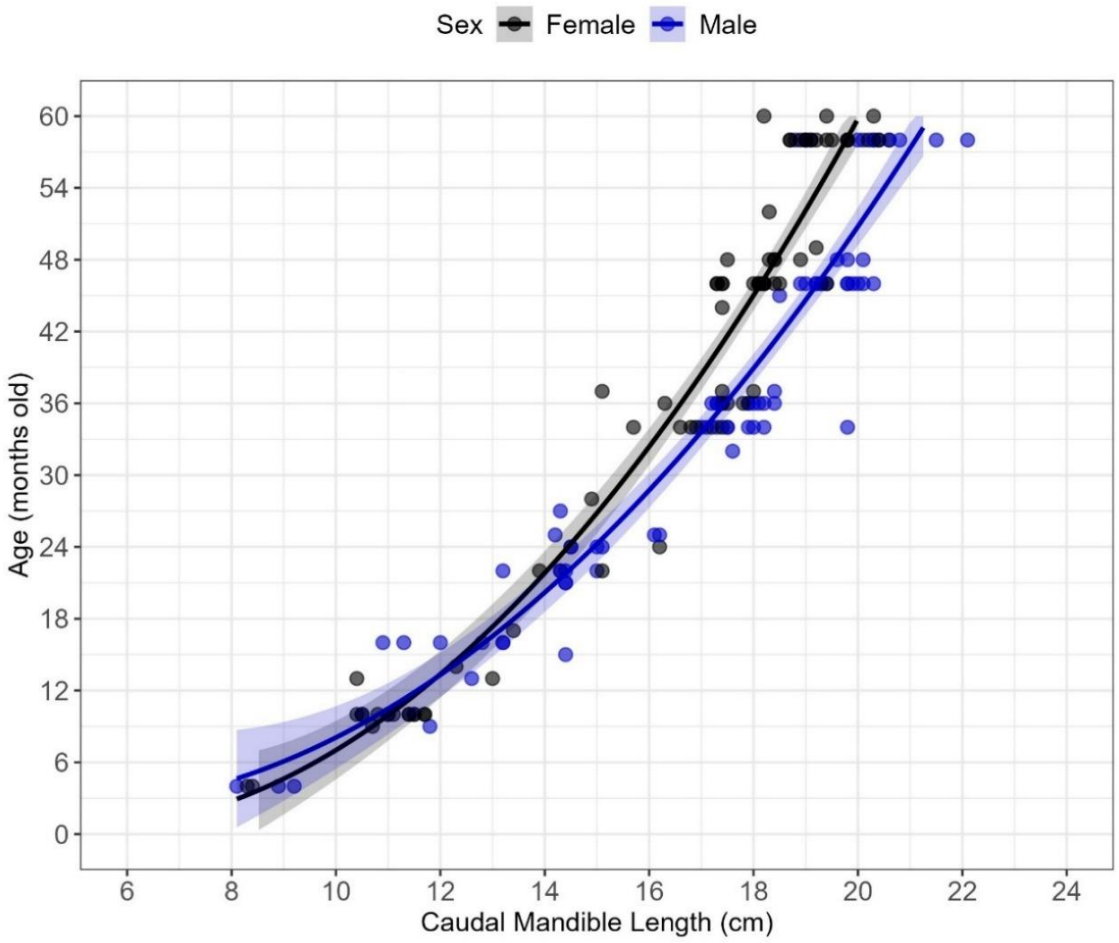




Statistical Analysis:

1. The linearity of relationships between predictors and age was assessed using scatter plots and residual plots. Collinearity among predictors was evaluated using the variance inflation factor (VIF).
2. When collinearity was detected, independent models were run to identify the best predictors of age. Molar heights (M2 and M3) and other variables not directly related to mandible morphometrics were excluded from analysis due to unavailability of measurements before eruption and the variability introduced by tooth wear pattern.
3. Model selection was performed using the Akaike Information Criterion (AIC) to identify the best-fitting models. Linear, quadratic, and interaction terms with sex were evaluated for each predictor. Quadratic terms were included to address non-linear patterns observed during initial visual assessments. Interaction terms with sex were tested to determine whether the relationships between predictors and age differed by sex.

Caudal mandible length was the best predictor of age but growth was found to slow before complete growth ceasation so a quadrartic model was necessary. Sex also influebce the models prediction of age indicating sex differences that need to be accounted for.
This process was also used for muskoxen from a single population but does clearly indicate the association between age and mandible growth.



Cementum Annuli Analysis

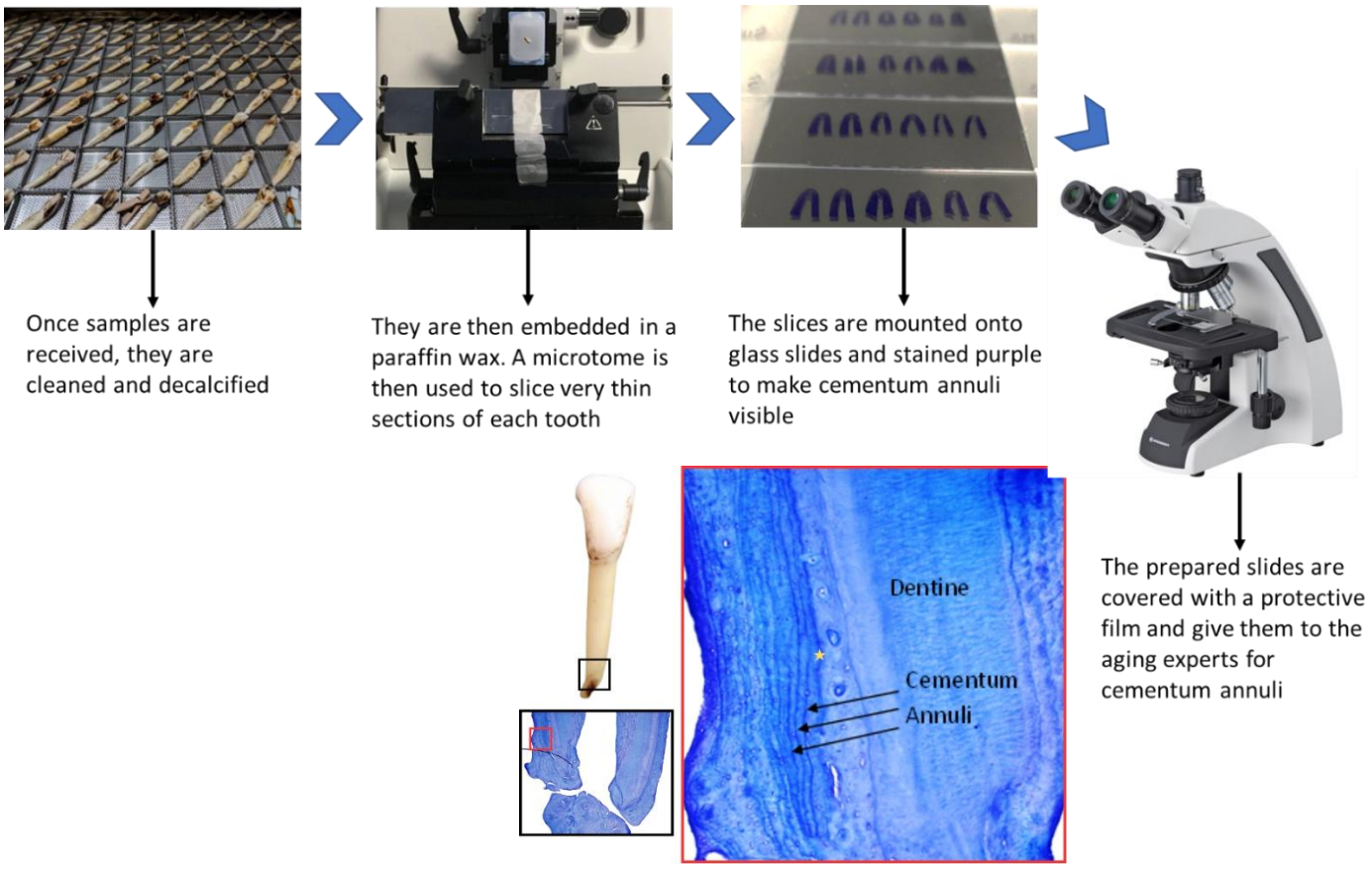
Extract the left I1 from muskoxen aged as 5^{+}yo or if examiner has uncertainty if animal's age from tooth eruption pattern. If left I1 is not available extract the right I1 or next available incisor (I2 left etc.).

1. Make two lateral cuts through the gingiva adjacent the I, ensuring not to damage the root.
2. Use a dental probe to loosen the attachment of the periodontal ligament.
3. Carefully use dental pliers and elevator to extract.
4. Place tooth in paper/coin envelope and label with the animal's ID number.

Lab Procedure for Cementum Annuli Analysis

1. Incisors are decalcified in hydrochloric acid, fixed in formalin, embedded using the paraffin method, and sectioned at 14µm using a microtome (Model RM 2155; Leica Biosystems, Buffalo Grove, IL, USA).
2. Each incisor gets sectioned was sectioned and mounted on slides, Giemsa stained (RICCA Chemical Co., Arlington, TX, USA), and cover slipped for microscopic examination of cementum annuli.
3. Next Matson's Laboratory North American muskox I1 model where the first defined annulus separated from the dentine is interpreted as year 3 and subsequent annuli are counted as observed. A birth date of May 1st is assumed, and the interpretation of the final cementum annuli was based on season or date of death.

Cementum Methods: Matson's Lab https://matsonslab.com/the-science/cementum-aging/



Muskoxen showed thin cementum annuli with little separaton between the growth and rest lines that while there was an experienced examiner assessing, these lines are not as distinct as seen in other species with high accuracy for cementum annuli age estimate. Therefore, an underestimated age is expected for muskoxen.