Jaw Examination
Standard Operating Procedure

Faculty/ Department or Institute/Centre	Faculty of Veterinay Medicine	Date Created:	
		Created By:	
Location		Revision #:	
		Revision Date:	July 2025
Supervisor		Revised By:	

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Authorization
Personnel processing jaws from muskoxen or caribou must have completed all required online biosafety training courses as well as a lab orientation. In case the jaws need to be processed in the biosafety working area of the necropsy room at Spyhill or the VSRS, additional specific orientation, rabies vaccine record and respirator fit test are required.

Hazards associated with equipment/machinery/materials/technique/process
The hazards associated with the jaw processing are zoonotic infectious agents that the samples may contain and wounds from handling a cutting knife. Some caribou and muskox populations from the Arctic have endemic infections of *Brucella suis* biovar 4.

Personal protective equipment
The protective equipment that working personnel should have are:
- Nitrille disposable gloves
- Cut-protective gloves when using cutting knifes
- N95 masks
- Protective glasses
- Scrubs
- Coveralls and/or apron

Materials needed
- Large calipers
- Scalpel blade and handle
- Forceps
- Necropsy knife
- Small and large whirl-paks
- Coin envelopes
- Dental elevator and extraction forceps
- *If extracting bone marrow:*
    - BOSCH reciprocating saw OR WORX oscillating multi-tool if extracting bone marrow; blades for reciprocating saw or oscillating multi-tool; and swivel vice
    - Disposable weighing dishes
    - Sterile swabs and cryovials
    - Electronic weigh scale capable of measuring to the nearest 0.01 g
    - Large flat plastic box with hole on the sides prepared specifically for bone marrow drying

Environment where task is to be undertaken
The procedure can be done in the VSRS Necropsy Lab, the dirty room of the HSC2535 lab, or in the “bio-bubble” at the DSU necropsy room in the Spyhill campus. However, when jaw samples come from populations known to be infected by *B. suis* biovar 4, the jaw processing needs to be performed in the bio-bubble at the necropsy room of Spyhill.

If working at DSU, the work must be finished 4pm at the latest and the workspace should be cleaned.

Before you start work
The lower jaw bones should be stored at -20°C until time of processing. The marrow fat estimate is based on the marrow water content, so it is important that the bone is not allowed to dry out. The jaws should be thawed before processing and analysis, but marrow extraction might be easier if the bone is still a little cold/chilly. The jaw bones should be removed from the freezer and left in a cool room to thaw overnight.

Emergency procedures
In case of personnel emergency (e.g. injury), follow the direction given to you during the introduction to the necropsy room. Alert the DSU or VSRS personnel present in the room if you require assistance. If no DSU or VSRS personnel present, use the phone in the necropsy room to contact personnel.
In case of general emergency (e.g. fire alarm), follow the direction given to you during the introduction tour.

Step by step procedures for task

Preparation

i. Use the datasheet provided (Appendix I) to enter the **Sample ID** provided by the submitter, including the UCID.
ii. Pre-label two small sized whirl-paks with UCID, animal ID, “muscle”, and either “Weston” or “DoD” (Department of Defence, Health Canada) for the two projects
iii. Pre-label a coin envelope with UCID, animal ID, and “i1 left” or “i1 right” (depending on if successful with extraction of left incisor). Preferrably i1 left to be extracted.
iv. If taking bone marrow: prepare two sterile swabs and pre-label two cryovials with UCID, animal ID, and either “Weston or “DoD”, and pre-label weigh boats with UCID and animal ID for bone marrow fat percentage.

Summary of samples to collect

i. 2x2 cm muscle sample in small whirl-pak to keep at -20°C for Weston
ii. 2x2 cm muscle sample in small whirl-pak to keep at -20°C for DoD
iii. 1 incisor tooth (i1) in paper envelope to keep at room temperature
iv. If collecting bone marrow, 2 swabs of the bone marrow in labelled cryovials to keep at -20°C for Weston or DoD

General procedure

1. Find where soft tissue is most plentiful and, if possible, remove two 2cm x 2cm pieces of muscle and place in sample bags to be frozen at -20°C for Weston and for DoD.
2. Remove all soft tissue from the jaw, including the posterior (hind) edge of the jaw, with a knife. The bone is fairly thin at the posterior edge, so be careful to not cut into the bone. Persons performing this task should use a cut-resistant protective glove in the opposite hand that uses the knife.
3. Take photos of the jaws as described in Appendix II.
4. Determine the age of the animal based on the teeth eruption pattern and according to the guide provided in Appendix IVa, IVb, and IVc. If muskox, refer to Appendix III for appropriate aging method.
5. Perform all jaw measurements **before** removing any teeth, as described in Appendix V, and note whether the jaw was dried or not.
6. Record incisor abnormalities and measures as described in the Appendix VI.
7. For both muskox and caribou jaws, extract an incisor (I1 left if possible) for aging by teeth cementum analysis, as described in Appendix VII. Use a tooth elevator to avoid any breakage of the teeth, as the root needs to be intact for this analysis. Place the extracted tooth into a paper envelop properly labeled with the ID of the animal, the UCID and the tooth identification (e.g. I1 left), and store it without sealing to let any minimal organic material dry out.
8. Marrow from the lower jaw can be extracted for analysis of water content, thereby providing a measure of fat content. Be sure all measurements have been conducted, photos taken, and incisor teeth extracted **before** marrow extraction. A detailed procedure is described in Appendix VIII.
    a. Take two sterile swab samples, detailed procedure described in Appendix VIII, and then extract remaining marrow for fat mass index.

Clean-up procedures

- All tools and surfaces shall be first scrubbed clean of tissue, fat, blood. Then disinfected using the disinfectant available in the necropsy room or the HSC2535 lab.

Waste disposal procedures

- Inorganic material should be disposed of in the bio-hazard bins available in the necropsy room
- Sharp metals (e.g. scalpel blades) must be disposed of in the bio-hazard container for sharp metals in the necropsy room
- Organic material processed in the DSU necropsy room are disposed of in blue barrels specifically for tissue disposal. Inorganic material may be disposed of in the yellow bio-hazard bin in the bio-bubble if not grossly contaminated with organic material.
- Organic and inorganic material in the VSRS necropsy room are disposed of in the red bio-hazard bin; the bag is then tied closed, the exterior is disinfected, and the bag is placed in the freezer just outside of the necropsy room.
- If the samples have been processed in the BSC in the necropsy room, all material (organic and non-organic) must be disposed of in the bio-hazard bin inside of the BSC in the necropsy room.

Maintenance

- Specify any periodic maintenance required for equipment.
- Identify who is required to do it and what documentation must be in place.

Signed:_____Date:_____
Supervisor

Appendix I: Jaw examination protocol form

*One form should be completed per jaw. *Caribou and moose versions available

Muskox Jaw Examination Protocol

Sample ID:_____ UCID:_____ CWHC n°:_____

Species:_____ Sex:_____ Age: ☐ Calf ☐ Yearling ☐ 2 yrs ☐ 3 yrs ☐ 4 yrs ☐ 5+ yrs Incisor Extracted: ☐ Yes ☐ No

Specimen: ☐ Whole Jaw ☐ Half Jaw ☐ Incisor Bar Condition: ☐ Fresh ☐ Bone Material: ☐ Physical Specimen ☐ Picture Collect Muscle: ☐ Yes ☐ No

ERUPTION PATTERN: ☐ All permanent teeth erupted **if not** D= Deciduous E= Erupting permanent P= Permanent

Molar 2 Height: _____ mm

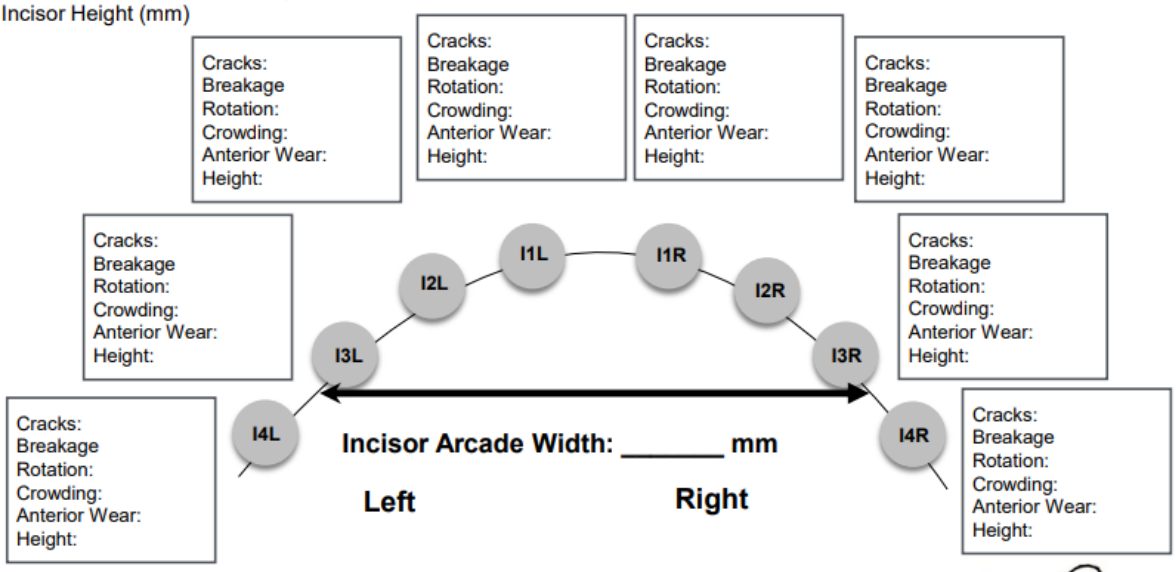
Molar 3 Height: _____ mm

I1	I2	I3	I4	P1	P2	P3	M1	M2	M3

Cusp: 1 2 3

INCISORS:

- Incisor Cracks (N=No, H=Horizontal, V=Vertical, HV=Horizontal and Vertical, NA=Tooth missing or broken under gumline)
- Incisor Breakage (N= No, <50%, >50%, 100%, PM=Post-Mortem)
- Incisor Rotation (N= No, 20˚, 45˚, 90˚)
- Crowded Incisor: (0%, 10%, 25%, 33%, 50%, 66%, 75%, 100%)
- Anterior Wear: (N=No, Y=Yes)
- Incisor Height (mm)



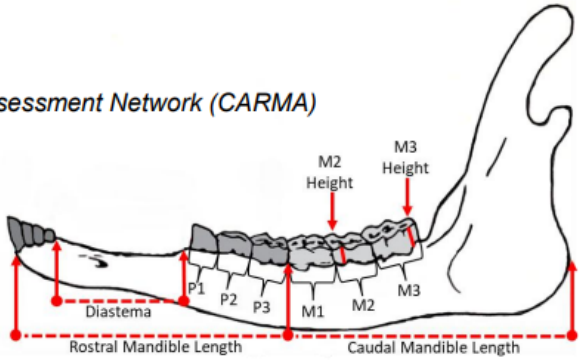
JAW MEASUREMENTS:

from the CircumArctic Rangifer Monitoring and Assessment Network (CARMA)

Diastema: _____cm

Rostral jaw bone: _____cm

Caudal jaw bone: _____cm



E. Suitor, F. Mavrot, S. Chopra, Kutz Lab, University of Calgary Updated 2024

Additional Findings: Teeth, Bone, Soft Tissues

Note all observations on illustrations below

Legend

- Food Impaction (FI)
- Diastema (DI) - Impacted/Not Impacted
- Missing (MI) - Impacted/Not Impacted
- Broken (BO)
- Mobile (MO)
- Swelling (SW)
- Draining Sinus (DS)
- Ulceration (UC)
- Vesicular Lesions (VL)
- Hyperemia (HP)
- Discolouration (DC)
- Abscess (AB)
- Necrosis(NS)
- Bleeding (BD)

Left

Right

Additional Remarks (* number on illustrations) :

Appendix II: Photo procedure of the jaws

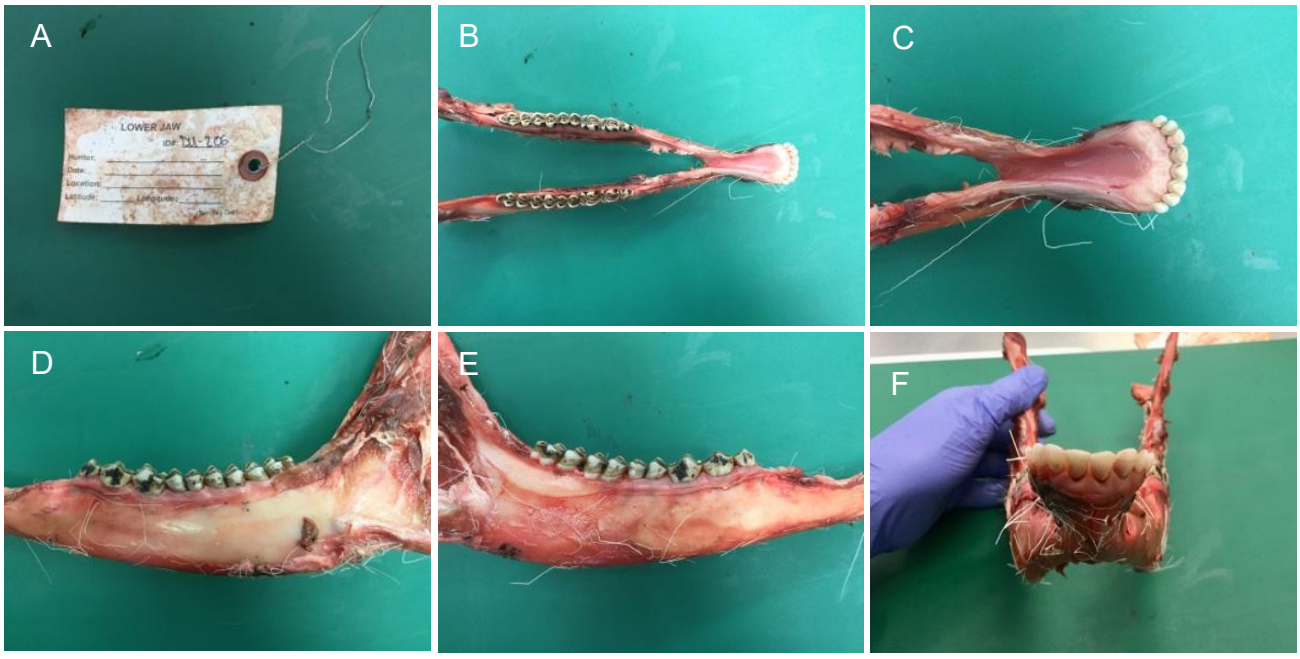
General instructions:

- Ensure you have taken the muscle samples for Weston and DoD projects
- Trim all excess tissue from the thawed jaw.
- Place your jaw on a dark (black) background to increase the picture quality.
- Make sure you have enough light in the room and avoid using camera flash. If this is not possible, check for reflections of the camera flash in teeth or wet surfaces that may affect picture quality.
- Camera flash can be reflected on teeth and wet surfaces and can decrease picture quality. Rather than using flash, make sure you have enough light in the room. If you use a flash, check that the pictures are OK.
- Make sure and review that your focus is on the surface of the teeth, not the gum or the background.
- Make an initial photo of the series with the animal ID or include a label with the animal ID in the first photo of the whole jaw.

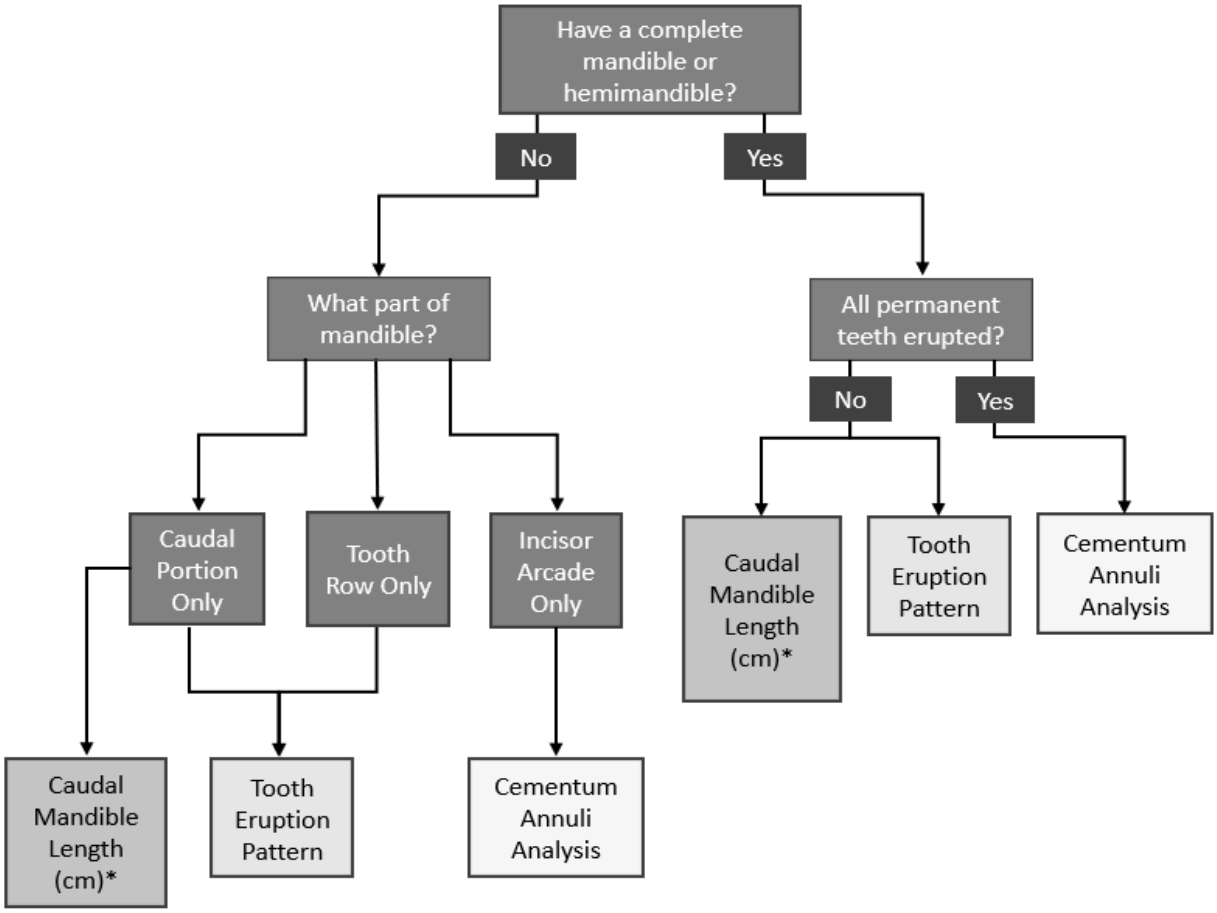
Take at least five pictures for archives, including a label with the animal ID next to the jaw:

- ID of the animal. You can include the ID next to the jaw as well (A)
- Whole jaw from the top view (B)
- Detail of incisors from the top (C)
- Lateral view of the molars and premolars (D and E)
- Front photo of the incisors (F)
- Any abnormal findings

Examples of photos:



Appendix III: Muskox aging method overview



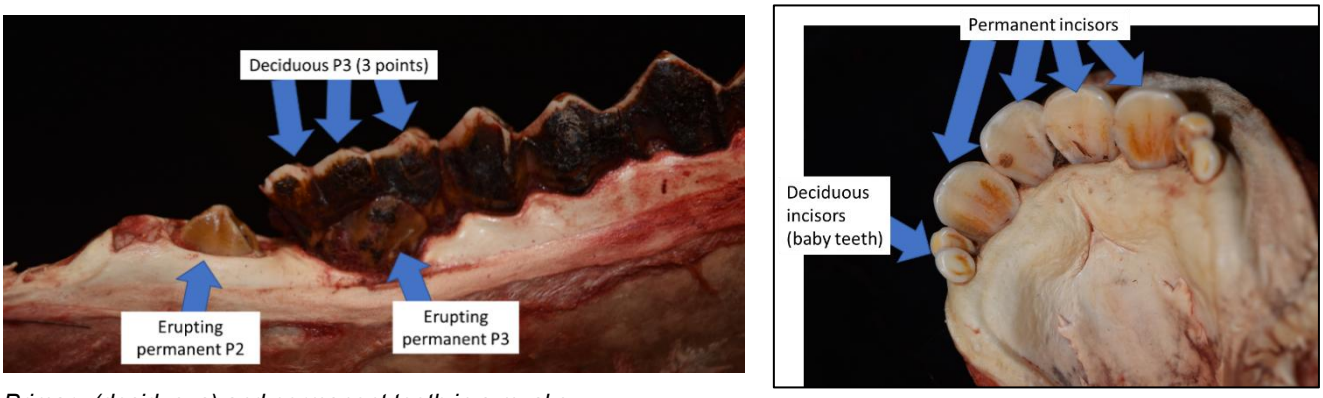
Overview of sample available and aging method. Figure created by Erica Suitor.

Appendix IVa: Age determination based on teeth eruption in muskoxen (Updated)

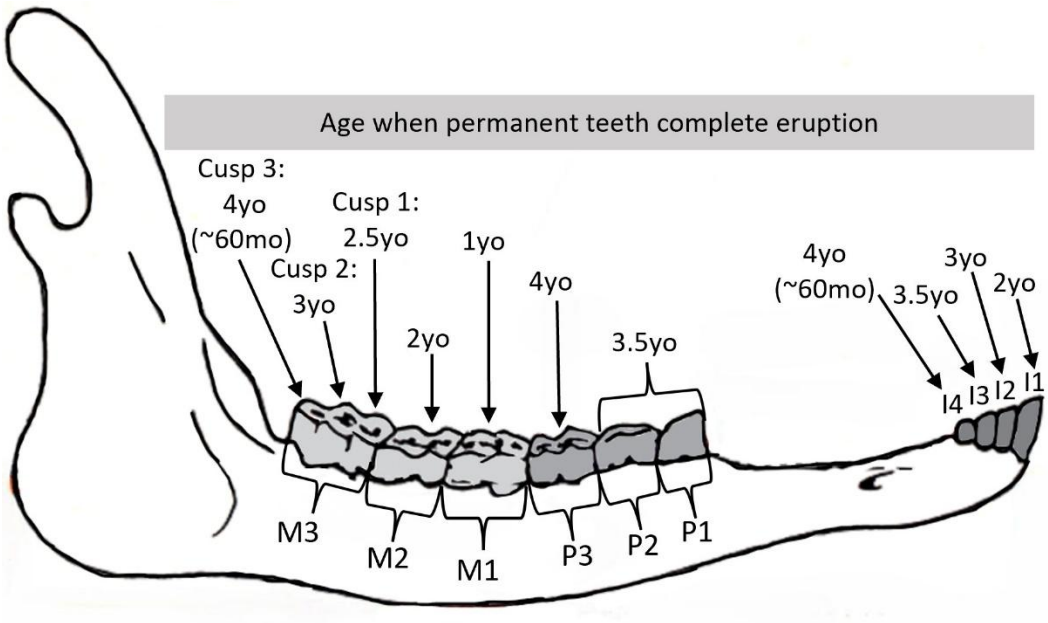
Muskoxen can be aged by tooth eruption up to 5 years old and can only be aged by month if month of death and an estimated birth date of May 1st is used. Eruption pattern can be recorded per tooth which can then correspond of their age.

Please note:

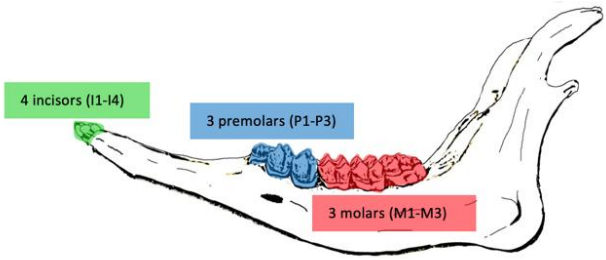
- Teeth eruption can vary between animals and may not correspond exactly to the guidelines presented here.
- Incisor 4 (I4) is sometimes missing or can erupt later than 4 years old; therefore, they are not always reliable to age.
- Primary premolars have one (pm1), two (pm2) and three (pm3) cusps (points). 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.

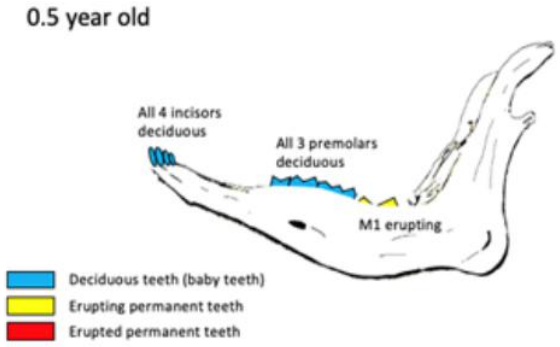


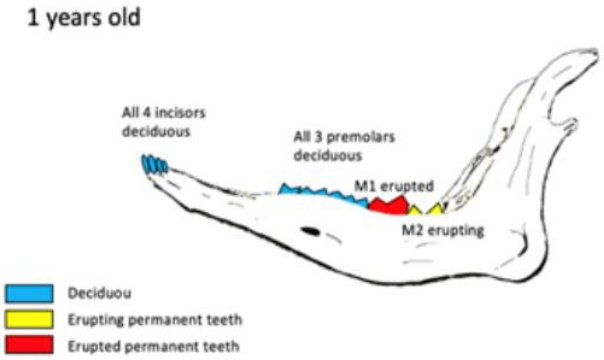
Primary (deciduous) and permanent teeth in a muskox.

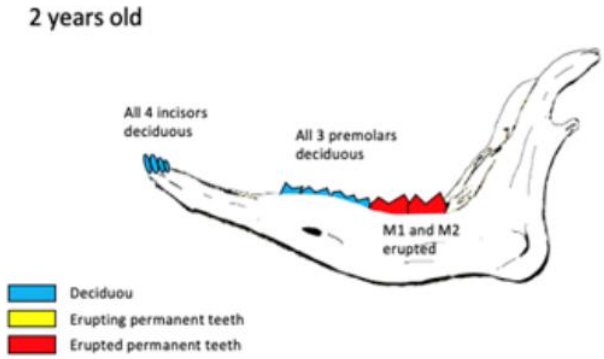


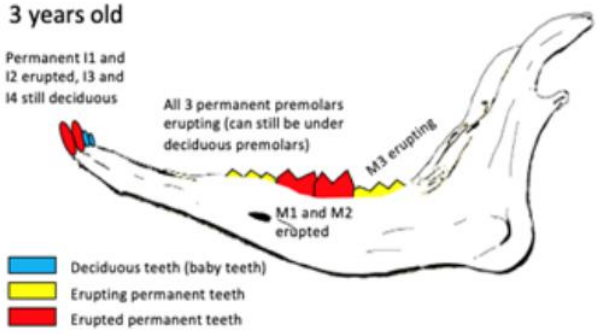
Adapted from Henrichsen and Grue, 1980, Age Criteria in the Muskox (*Ovibos moschatus*) from Greenland, Danish Review of Game Biology Vol. 11 Nr. 4.

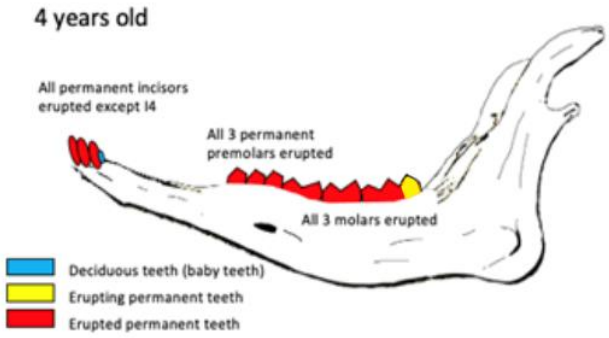


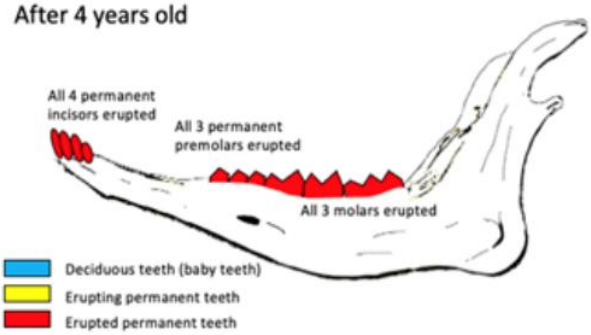












Appendix IVb: Age determination based on teeth eruption in caribou

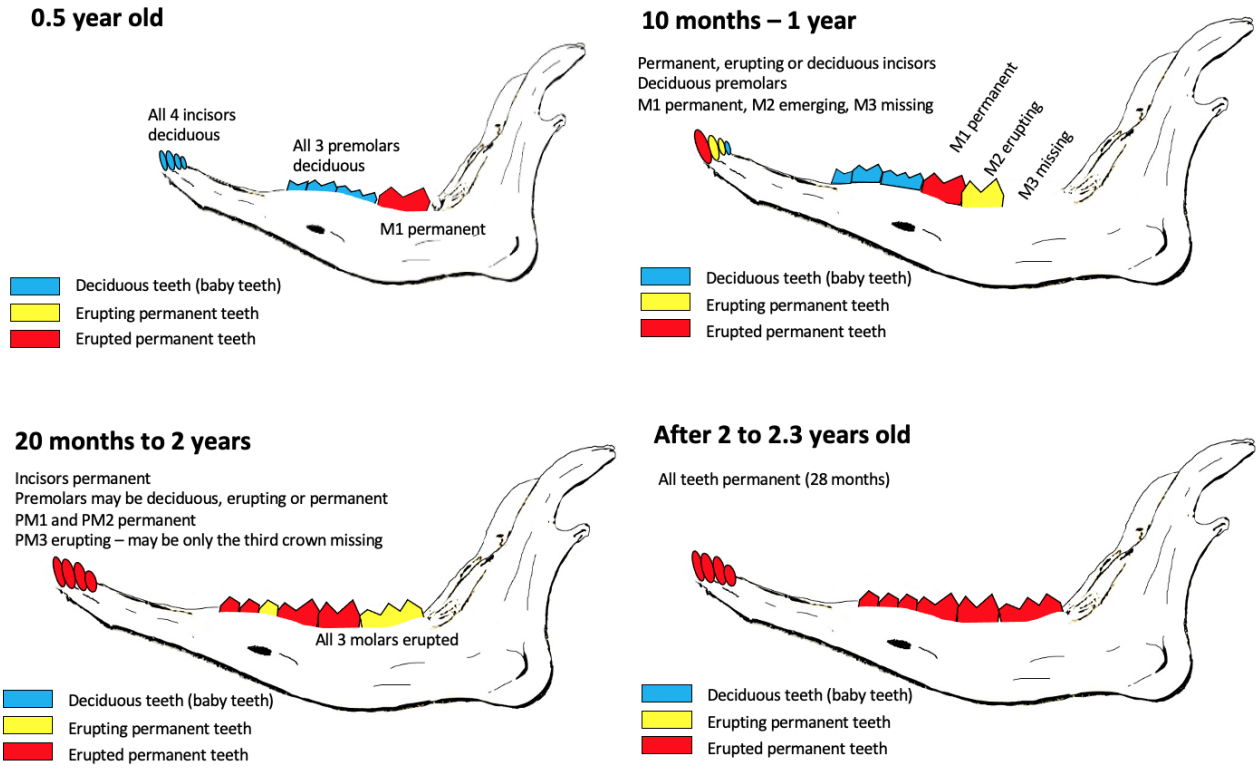
For a detailed description of teeth eruption pattern in *Rangifer* see Frank L. Miller 1992 - Eruption and Attrition of Mandibular Teeth in Barren-Ground Caribou. The Journal of Wildlife Management. Vol. 36 (2), pp. 606-612.

Please note:

- Teeth eruption can vary between animals and may not correspond exactly to the guidelines presented here. Incisors are found to vary more than molars.
- Teeth not fully erupted but >50% may be difficult to distinguish from a fully erupted teeth, use other evidence to estimate the age in case of doubt.
- Primary PM3 has three crowns and permanent PM3 has two crowns.
- For non-adult individuals (M3 missing or not fully erupted) teeth worn indicates primary teeth.
- For the closest calculation of the age of the animals, consider the date of sampling assuming the 1st of May as the standard date of birth.

Age categories according to the CARMA protocol:

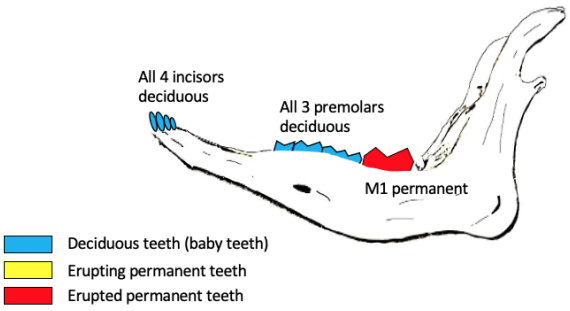
- Calf: from 0 to 1 year old. All teeth are primary but M1 is completely erupted at 5 months, M2 may be erupting from 10 months onwards and incisors from 9 to 11 months. M3 is missing.
- Subadult: from 1 to 3 years old. It includes yearlings (from 1 to 2 years). M3 may start erupting around 15 months and completes at 28 months. Premolars erupt later, at around 21 months, but they are completely erupted at a similar age than M3 at 28 months old.
- Adult: from 3 years old onwards. All teeth are permanent.

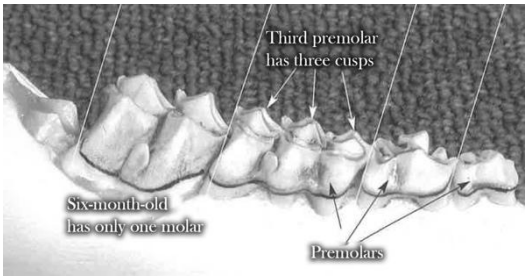


Appendix IVc: Age determination based on teeth eruption in moose

Adapted from North Dakota Game and Fish Department (https://gf.nd.gov/hunting/aging-big-game/moose)

0.5 year old

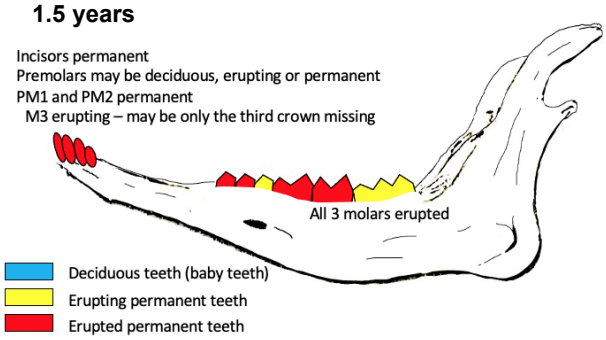




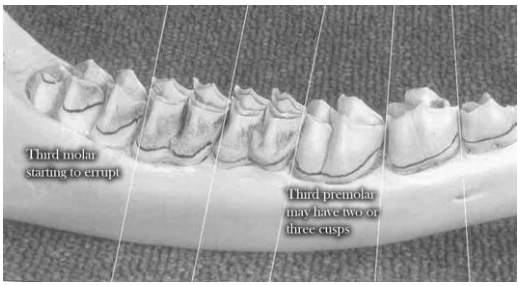
All deciduous incisors are present. Only premolars and first molar are present. The third premolar has three cusps (deciduous).

1.5 years

Incisors permanent
Premolars may be deciduous, erupting or permanent
PM1 and PM2 permanent
M3 erupting – may be only the third crown missing

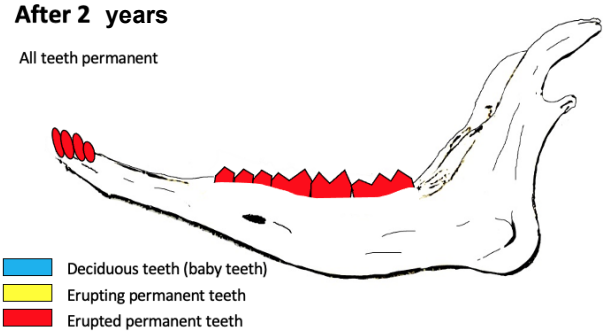


All permanent incisors, may have erupting I4. Six cheek teeth are visible. The third premolar may still have three cusps and be well worn. Third molar starting to erupt through the gum and shows no sign of wear. Lingual crest of molars have sharp points.

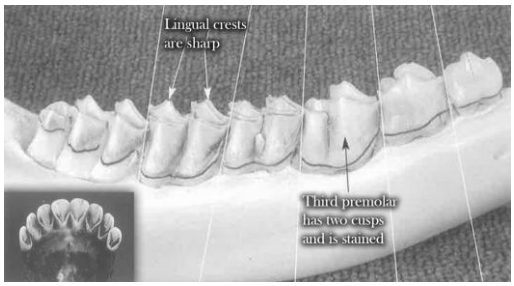


After 2 years

All teeth permanent



Last cusp of third molar erupted and in place. All the premolars and molars show wear and are stained. All incisors are in their final position.

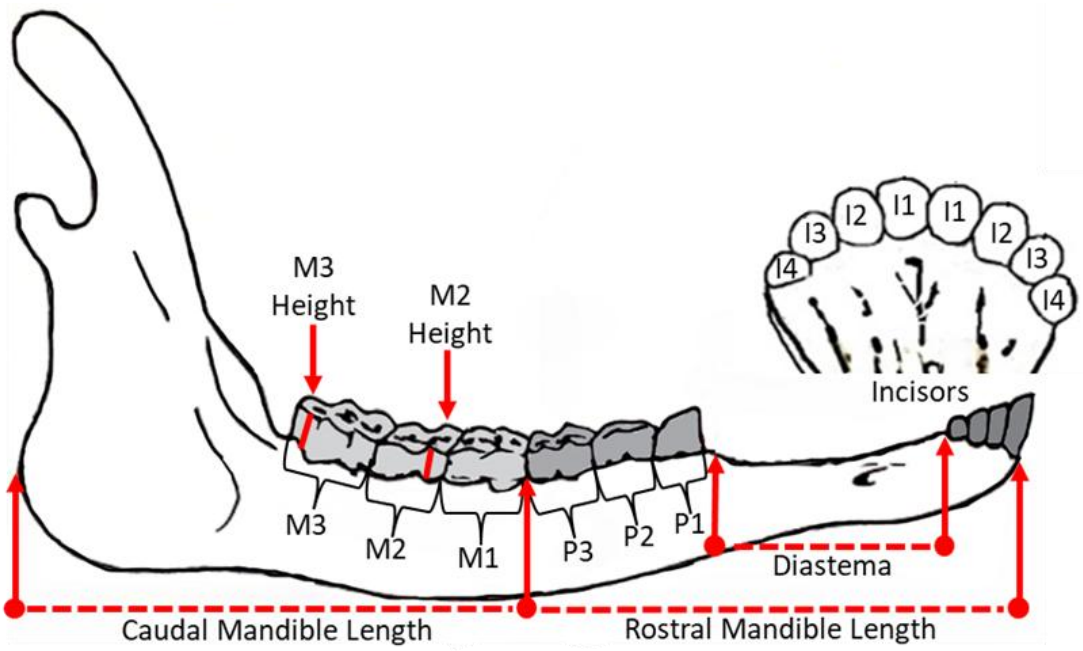


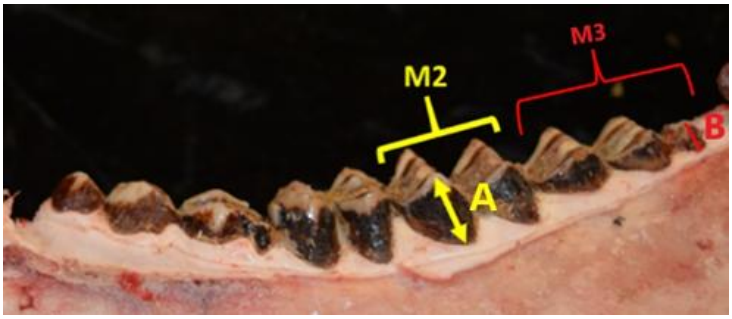
Appendix V: Mandible morphometrics

(Adapted from the CWHC protocol)

Use calipers, when possible, to measure to the nearest millimeter (mm) the following, as illustrated below:

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



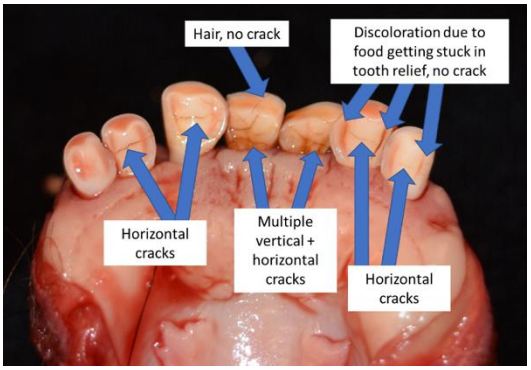


Appendix VI: Incisor abnormalities and additional measures of incisors

Annotate all findings in the corresponding place in the datasheet form.

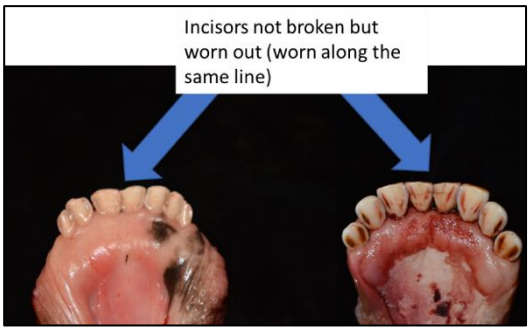
Cracks on incisors:
Record all cracks on both side of each incisor:

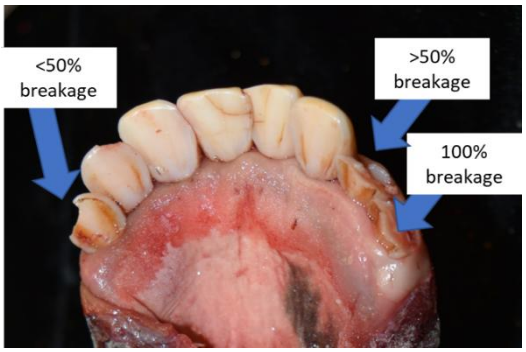
- Horizontal cracks (H)
- Vertical cracks (V)
- Horizontal and vertical cracks (HV)
- No cracks (N)

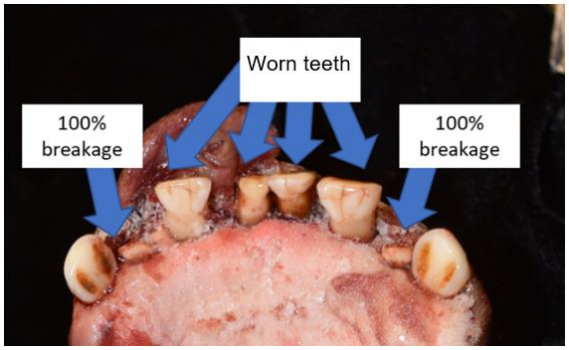


Incisor breakage:
Record any breakage of incisor teeth based on the visible tooth

- (No) no breakage
- (< 50%) of visible teeth
- (>50%) of visible teeth
- (100%) - under gum line
- PM- Post-mortem breakage
- NA – missing (Note: sometimes it is difficult to distinguish between a missing tooth and a tooth broken under the gum line. You can cut in the gum to find the root of the tooth. In case of doubt, put a note on the examination sheet.)

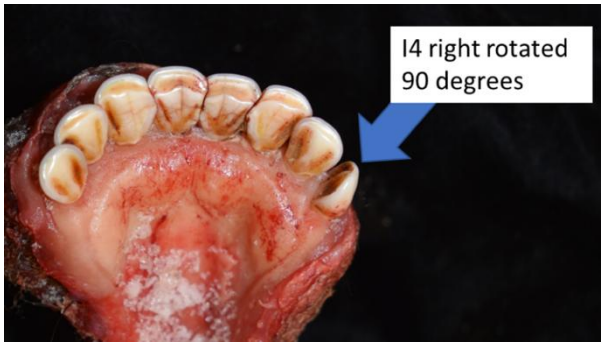






Incisor rotation:
Record if any incisor is abnormally rotated. Note the degree of rotation as follow:

0˚, 20˚, 45˚, 90˚



Incisor misalignment:
Record all further misalignments on incisors where teeth are protruding labially or lingually. This is not common so can be noted under abnormalities

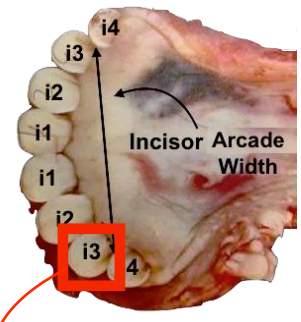
Anterior Wear:
Look at the labial face of the incisors to see if there is any wear. Looks like a scraping motion would cause this type of wear.

Incisor Crowding:
From lingual view of tooth: what percent of the tooth overlaps with an adjacent tooth. Measured as 0%, 10%, 25%, 33%, 50%, 66%, 75%, 100%.

Displacement: Note if any of the teeth look like they have moved from their position.

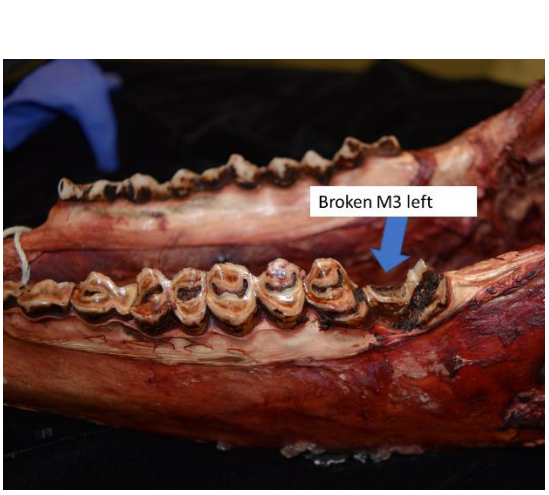
Incisor height: Measure the height (mm) of each incisor from the cervical point between the root and crown to the tip of the crown.

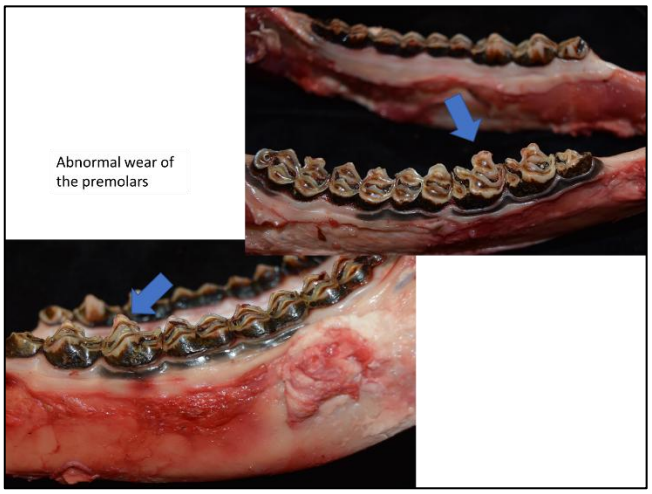
Incisor arcade width:
Measure the length (mm) between the outside edges of the left and right i3 at the gum line level.

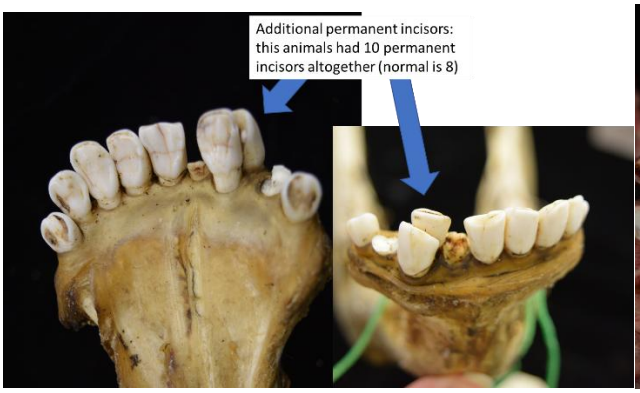


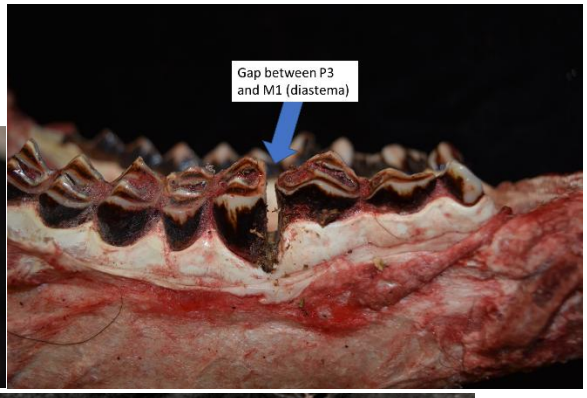
Additional findings:
Examine the rest of the jaw and record any abnormality. Try to move the premolars and molars. Look at possible asymmetries between left and right. Inspect not only the teeth but also the gum.

Some examples of abnormal findings in muskox jaws:









Appendix VII: Incisor extraction for cementum annuli analysis

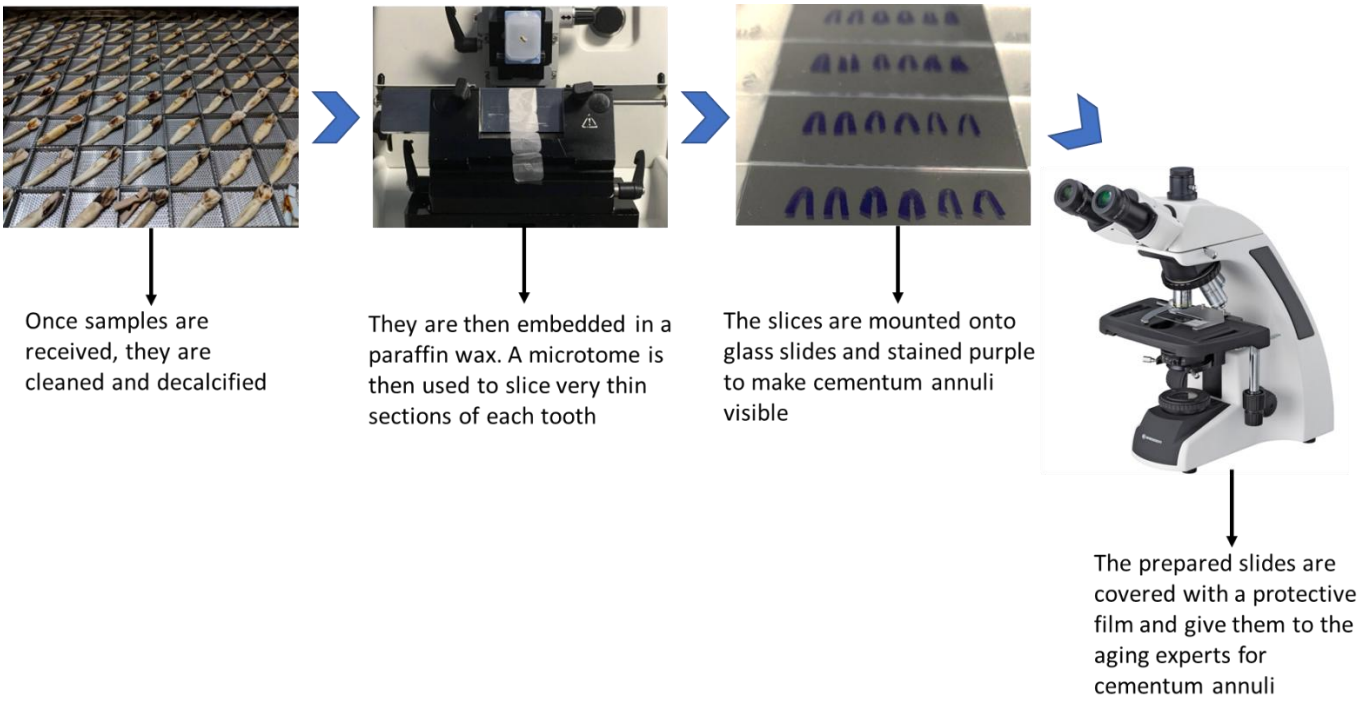
Incisor Extraction
Extract the left I1 from muskoxen aged as 5⁺yo and caribou aged as 2⁺ yo, or if examiner has uncertainty of animal's age from tooth eruption pattern. If left I1 is not available, extract the right I1 or next available incisor (I2 left etc.).

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

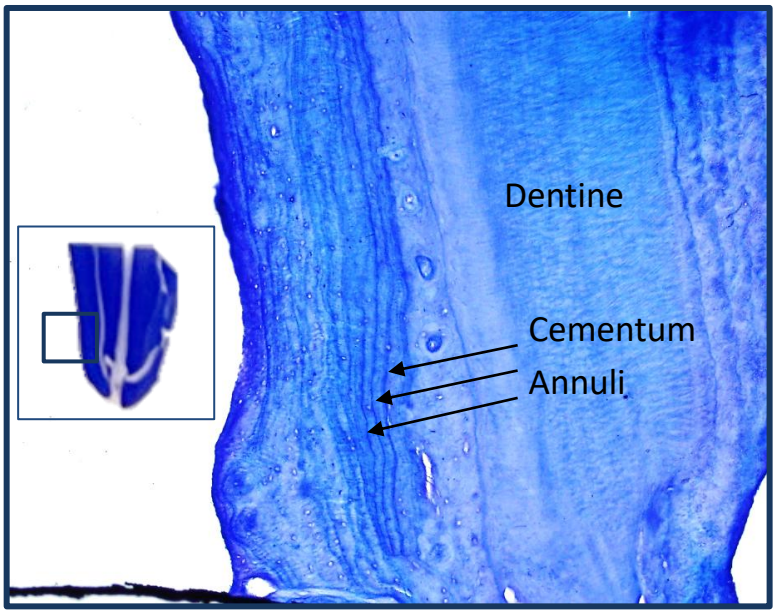
*Lab Procedure for Cementum Annuli Analysis (**not done during processing**)*

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 is sectioned and mounted on slides, Giemsa stained (RICCA Chemical Co., Arlington, TX, USA), and cover slipped for microscopic examination of cementum annuli.
3. Slides are then examined.
    a. Matson's Laboratory North American muskox I1 model: 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 is based on season or date of death.

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



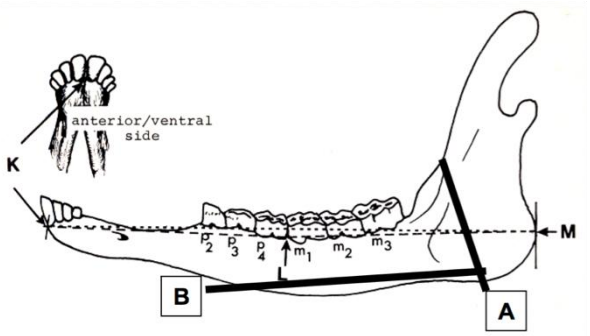
Note: Muskoxen showed thin cementum annuli with little separaton between the growth and rest lines and, while there was an experienced examiner assessing, these lines are not as distinct as seen in other species with high accuracy for cementum annuli age estimate. Therefore, an underestimated age is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



Appendix VIII: Lower jaw bone marrow extraction

(Adapted from the CWHC SOP procedure)

1. Prepare and label each individual weigh boat with the **UCID** and **Sample ID** numbers of each animal using a Sharpie marker on the outside bottom or side of the weigh boat. Record the weight of each empty weigh boat to the nearest 0.1 g. Use the bone marrow weight data sheet (Appendix III).
2. Additionally, prepare and label two cryovials with UCID, sample ID, and “Weston” or “DoD” for sub-samples of the bone marrow.
3. Ensure that the **blade is disinfected between bones**.
4. Saw completely through the jaw for marrow extraction at line A (see diagram).
5. Saw along the outside of the jaw at line B.
6. Crack jaw open with a sturdy knife or screwdriver.
7. When taking samples for brucella testing (Weston) and DoD:
   i. Flame the open end of the bone.
   ii. Take two swabs of the bone marrow after flaming and place in pre-labelled cryovials. Store frozen at -20C°.



8. Extract marrow using lab spatula or dull carving tool. Be careful to exclude any bone fragments from the inner jaw – you will be able to feel these with your fingers. Try and collect a large enough portion of marrow to allow for calculation of proportion of water in the sample (wet versus dry weight; 5-10 g).
9. Place the marrow of each jaw into its corresponding container. Weigh each container with its marrow and record the weight to the nearest 0.1 g.

   IMPORTANT: Clean any bone marrow spill that may happen, especially on the vessel area where the ID is written. The ID with the marker will disappear if it is not removed immediately.

10. If acces to an oven, dry the marrow in containers (if heat tolerant/metal) in an 85°C oven. Check and record the weight at 24 and 48 hours before samples are fully cooled. If the weights are different, place the container back in the oven for another 12 to 24 hours (for total drying time of 72 hours or more if needed). When the weights recorded are consistent/staying the same, drying is complete.
11. If no oven, place weigh boats in the large flat plastic box with holes on the sides prepared specifically for bone marrow drying. Leave box to dry for minimum one week.
12. After one week, begin weighing the marrow daily and record the weights on the datasheet. When the weights recorded are consistent/no longer declining, drying is complete.
13. Subtract the weight of the vessel/container from the weight of the marrow (once weights are consistent) to give an adjusted marrow weight. Take final adjusted marrow weight and divide it by the initial adjusted marrow weight to give the percent jaw marrow fat, an index of animal condition.
    i. Weights of the bone marrow at the first and last weighing:
       Weight of the bone marrow and dish together - weight of empty dish = Bone marrow weight
    ii. Percent marrow fat:
       100 x (bone marrow weight at the end / bone marrow weight at the start) = Percentage fat in the bone marrow
14. Once you have obtained the results for each sample, dispose of the weighing dishes and the bone marrow in the yellow biohazard bin.

Appendix IX: Canadian Wildlife Health Cooperative specimen submission form

CANADIAN WILDLIFE HEALTH COOPERATIVE

SPECIMEN SUBMISSION FORM

ALBERTA REGION
Faculty of Veterinary Medicine, University of Calgary
11877, 85th Street, NW Calgary, AB, T3R 1J3
403.220.2806 dsu@ucalgary.ca

CWHC OFFICE USE ONLY

CWHC # _____ For research use? _____ Grant # _____

DSU Case # _____ PI _____

SUBMITTER INFORMATION

Organization _____ Address

Name _____

Phone number _____

Email address _____ Fax number _____

FINDER INFORMATION (If different from above)

Name _____ Address

Phone number _____

Email address _____ Copy report to _____ (Name and either email, fax, or address)

SPECIMEN INFORMATION

Species _____ Specimen ID/Reference # _____

Date found or collected _____ # Submitted _____

Date of death (if known) _____ Total dead ____ Total sick ____ Total healthy ____

Whole carcass or portion? _____ Age _____ Sex _____

How found? _____ (Dead/alive & died/shot/trapped/angled/netted etc.) If held in captivity; for how long? _____

How euthanized? _____ How were samples stored? _____ (Fresh/cool/frozen/fixed etc.)

LOCATION WHERE SPECIMEN WAS FOUND

Address/Location description (Please be specific. Enter legal land description here if known) _____

Latitude _____

Longitude _____

GPS Coordinates (please use degree decimal WGS84 setting & four decimal places)

or UTM Coordinates ____ _____ _____

ADDITIONAL DETAILS

Clinical signs such as: unusual behaviour and physical appearance, environmental circumstances (land use, habitat types, agricultural practices, proximity to roads or power lines, potential for poisoning etc.), climatic factors, suspected diseases, was the animal treated for disease, were samples sent to a lab (if so, which lab?). Where multiple animals/species are involved, please list total dead/sick/healthy by species if known. If samples were collected and sent to a lab, what type of sample and which lab?

Affiliated to Kutz Research Group (contact before tissue disposal and secondary use of tissues and analyses results).

NOTE: If you need more space, please use the other side of this page.

Please note: submission of this form signifies permission for the retention and use of the personal information contained herein for the purposes of correspondence, follow-up investigation, reporting of results, and geographical analysis of incidents.

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www.healthywildlife.ca
www.cwhc-rcsf.ca