# APPENDIX V

# AGING TECHNIQUES

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I. INTRODUCTION –

Two methods are commonly applied to age big and trophy game, and furbearing animals. One is based on tooth replacement and wear, and the second on cementum annuli deposition in roots of incisors, canines, and premolar teeth. The replacement and wear techniques are used to age game animals in the field. Aging based on cementum annular rings is more accurate, but requires extraction of a tooth for laboratory processing.

II. FIELD TECHNIQUES FOR AGING –

A. Deer, Elk and Pronghorn –

Pojar (1997) described field techniques for aging deer, elk, and pronghorn (attached). Most age data are collected during hunting seasons, when large samples of harvested animals are available. Some individual variation results from a natural range of birth dates and genetic differences. Based on tooth replacement, wildlife managers can assign animals to age categories through the maximum age at which all deciduous teeth are replaced. Tooth wear criteria enable managers to place individuals into several year classes extending beyond the age at which permanent dentition is acquired. However, diet and soil conditions contribute to significant geographic variation in the rate and degree of tooth wear for any species (Dimmick and Pelton 1996). Based on Pojar's criteria, it is possible to age pronghorn up to 4.5 years, and mule, white-tailed deer and elk to 3.5 years. Tooth replacement in white-tailed deer is very similar to that of mule deer, except white-tails acquire a complete set of permanent dentition by age 20-24 months, the process in not completed until 24-30 months for mule deer.

B. Moose –

Deciduous teeth are replaced more rapidly in moose than in deer or elk. The process is completed by about 19 months of age. Criteria for aging older animals are based on measuring the height and width of the buccal teeth and the length of the jaw. This may not be practical in field situations, so aging based on cementum annular deposits in the first incisors is recommended.

C. Black Bear and Grizzly Bear –

Black bears acquire a complete, permanent dentition by two years (Marks 1966) and is probably true of grizzly bears, as well (Mundy 1964). Older bears are aged

based on cementum annular deposits in the upper premolar teeth. These teeth are easily extracted from live bears or carcasses without harm or mutilation of the animal. Roots of many lower premolars are broken in the socket, and this interferes with accurate aging based on cementum annuli.

D. Bobcats and Lynx –

Tooth replacement criteria are applicable to bobcats up to 240 days (Jackson 1988) and believed similarly applicable to lynx. Permanent teeth are acquired during the first winter. Kittens are distinguished from juveniles or adults based on body size, presents of spots, and tooth wear. Ages of older animals are based on cementum annuli. The foramen of the canine tooth closes at 13-14 months; however, kittens from late litters may retain open root canals during early months of their second winter. This could cause some miss-classifications of kittens and juveniles.

E. Mountain Lions –

Lions can be separated into three age classes based on weight, pelage characteristics, tooth eruption and wear, and tissue changes that indicate breeding by females (Lindsey 1987). Anderson (2000) developed criteria to estimate lion age classes based on body size, dentition, and presence of spots and bars on the pelage. These characteristics are depicted in Figs. 1-6, and are summarized below:

1..Estimating Mountain Lion Age Classes –
Reliability: (1) teeth, (2) bars, (3) spots

a. Kitten (5-6 months, Fig. 1) –
   i. At 4 months deciduous canines are fully erupted (10 mm). Permanent canines begin to erupt at 6-8 months and are fully erupted at 15-16 months.
   ii. Penis sheath is dark. Spots are present on hind leg, bar on foreleg.
   iii. Faded spots are present on outer forelegs. Faint spots are visible elsewhere on upper body.

b. Subadult (1.5-2.5 years, Fig 2) –
   i. Canine teeth are fully erupted (males>26 mm, females 22-25 mm); teeth are white and sharp, no canine ridge is present.
   ii. Bars are present on forelegs, spots inside hind legs. Penis sheath is dark.

c. Young Adult (2.5-4 years, Fig. 3)
   i. Teeth are slightly stained, very little wear is evident; canine ridge appears just below gums.

ii. Spots are present inside hind legs and bars on forelegs. Vulva spot on females is dark.

d. Older Adult (4+ years, Fig. 4)

i. Teeth are well stained, canine wear is evident, outer incisors are worn almost even with other incisors.
ii. Canine ridge is obvious and well below gums (4 mm).
iii. No spots on bars are evident on white fur of belly or legs.

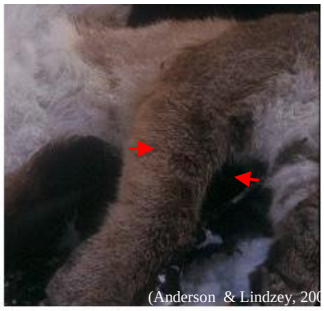
e. Additional examples of tooth eruption, staining, and wear are depicted in Figs. 5 and 6.

f. Differences in male and female genital spots are shown in Fig. 7, and nipple size and shape for lactation status are shown in Fig. 8

g. Cementum annular deposits are not currently useful for aging mountain lions (Lindsey 1987). The technique is still under development. Matson (1996) reports cementum annuli are relatively indistinct. Cementum thickness, indistinct and inconsistent staining of annuli, and experience of technicians affect the accuracy. Matson rates the method as a little more than approximate. However, age results from Matson's lab and the Wyoming Game and Fish Lab have been comparable and are useful for confirming general age classes (Moore 2004, pers. commun.).


Note deciduous canines. Fully erupted at ~4months (10mm). Permanent canines begin to erupt at 6-8 months and are fully erupted at 15-16 months.


Note dark penis sheath, hind leg spots, and bar on foreleg (arrows).




Note faded spots on outer forelegs. Faint spots present elsewhere on upper body for this age class.

Fig. 1. Kitten (<12 months).


Subadult male (1.5-2.5 yrs). Canine teeth fully erupted (males >28mm, females 22-25mm); teeth white, sharp, no canine ridge.


Note bars on forelegs, spots inside hind legs, and dark penis sheath (arrows).

Fig. 2. Subadult (1-2.5 years).


Young Adult Female (3-4 yrs). Teeth slightly stained with very little wear. Canine ridge just below gums (arrows).


Note spots inside hind legs (presence variable) and bars on forelegs. Dark vulva spot is out of view.

Fig. 3. Young adult (3-4 years).

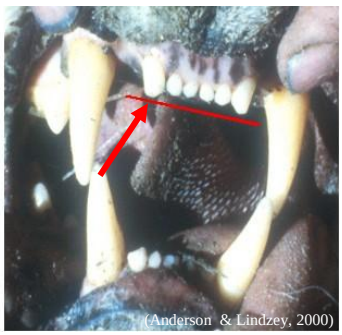

Older Adult Female (>4 yrs). Teeth well stained, canine wear evident, outer incisors worn almost even with other incisors (arrow). Canine ridge obvious (pen tip) and well below gums (≥4mm).


No spots or bars evident on white under-fur; bars may be present, but faint.

Fig. 4. Older adult (> 4years).


All permanent teeth erupted. Canines 1/3-1/2 extended. Note presence of deciduous canines. Known age: 9 months.

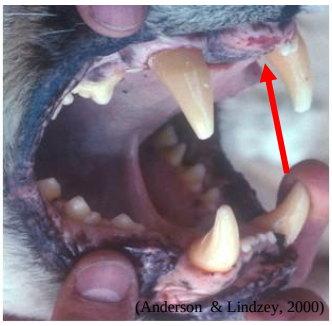

Teeth slightly stained with no wear. Canine ridge absent. Est. age: 1.5-2.5 (both sexes). If canine ridge present: female 2-3, male 3-4.




Teeth moderately stained with slight wear. Outer incisors worn at tips, canine ridge present just below gum line. Est. age: female 3-4, male 3-4 if any spotting/bars evident, 5-6 otherwise.

Fig. 5. Additional examples of tooth eruption, staining, and wear (note incisor curvature changes from concave to convex with age).


Notable wear and staining. Canines worn at tips, upper incisors worn close to gums with outer incisors worn nearly even with others. Canine ridge well below gum line (about 4mm). Est age: female 5-6, male 7-9.




Excessive wear and staining. 20% of canines worn off. Canine ridge well below gum line (>4mm). Incisors worn even or missing. Est. age: female 7-9, male 10+.


Most teeth worn to gum line with dark staining. Estimated age: 10+.

Fig. 6. Additional examples of staining and wear, continued.


Female (~1" from anus)


Male (4-5" from anus)

Fig. 7. Male and Female Genital Spots.


Has never lactated
(~4-5mm wide)


Has previously lactated
(~8mm wide)

Fig. 8. Nipple size and shape relative to lactation status. First lactation typically occurs at 2.5 years.

## III. LABORATORY TECHNIQUES BASED ON CEMENTUM ANNULI –

Matson (1996) reports the status of the cementum annuli technique applied to 24 species of North American Mammals. He describes the standard tooth to collect from each species, criteria for identification of juveniles, clarity of cementum patterns, accuracy of the method applied to each species, and the amount of experience technicians need to competently analyze tooth sections. Cementum annuli are present in virtually all mammals. The technique is recommended for aging deer and elk older than 3.5 years, pronghorn older than 4.5 years and moose older than 1 year. It is the preferred method for aging bears and bobcats. However, the cementum technique is unnecessary when ages of younger mammals can be easily interpreted from the presence of deciduous teeth, thin root walls, open root tips, and sharp occulsal surfaces on incisors.

Cementum is deposited as layers on the roots of teeth each year. In cross-sectional view the bands close to the dentine are from earlier years and the current-year layer is near the exterior surface of the root. The animal's age is determined by counting annular layers. The annuli appear as narrow, darkly stained bands separated by broader, weakly stained bands. The age of a mule deer, for instance, is usually one more year than the number of dark annuli in the first permanent incisor. In most longitudinal root sections, the cementum annuli should be clearly visible. However, examine all of the cementum carefully as areas of resorption or tooth repair may be present, resulting in fewer annuli or false doublets or triplets. Other physiological stresses such as rut, diet, or estrus can also produce false annuli. Tracing the annuli

around the entire root section and observing where doublets or triplets separate and come back together can minimize the potential for error.

A. Collection of Teeth –

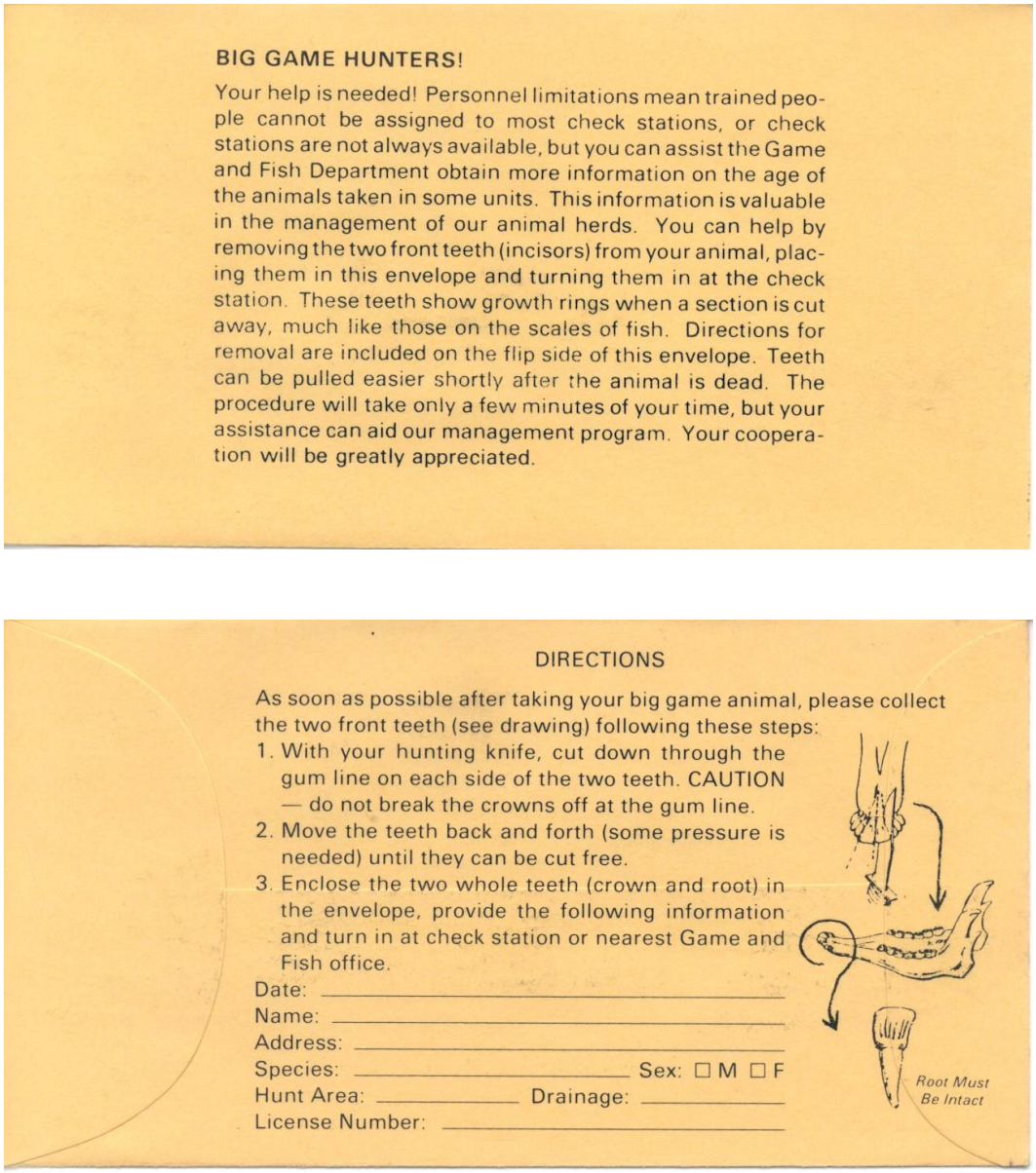
The following teeth are collected to age various species: Ungulates – the two central incisors; bears – the upper first premolar; cougars – the second upper premolar; and bobcats/lynx – the canine. The cementum of most ungulates is thickest on the anterior and posterior portions of the root tip. A 10 mm portion of the root that includes the tip is most useful for accurate age determination. Avoid breaking the root when the teeth are extracted. For ungulate teeth, use a knife to cut the gum tissue between the two central incisors and on each lateral side. Twist while pulling, to facilitate extraction (see attached tooth collection envelope diagram). Premolars can be removed with a dental elevator. If the premolar is taken from a carcass, cut the gum line from all sides of the root. Clasp the tooth with pliers and twist while pulling it. To remove canines, the jaw must be heated to 60-80º C for up to 12 hours before pulling. Use a bone hacksaw to cut off the anterior portion of bobcat jaws. Be sure not to damage the root of the canine. Jaw sections are then tagged and submitted to the lab where they are immersed in water and autoclaved for a short time to loosen the canines. Both canines are removed from the heated jaw with pliers by twisting and pulling. Teeth are stored in pre-labeled paper envelopes or tooth boxes. Do not store teeth in plastic bags or wrap because the plastic seals moisture in that leads to spoilage.

Teeth are solicited from big game hunters who extract and enclose them in postage-paid mailers with instructions. These are issued with the licenses. When an animal is killed, the hunter removes a tooth and mails it in the box supplied by Biological Services. Examples of the label and instructions for hunters are attached (Tooth Collection Envelopes, Fig. 9). The hunter is asked to provide the following information: name, complete address, date of kill, species, sex (M/F), hunt area and drainage. The department returns a notification of the animal's age to each hunter who submits teeth. Hunters are interested to learn the ages of animals they harvest. The notification is additional incentive to submit teeth for age determination

B. Laboratory Processing –

All teeth delivered to the laboratory are sorted according to species and assigned a unique identification number. The information on the envelope/box is recorded in a computer database. Packages containing elk, moose and bison teeth are opened, incisors are separated with bone shears, and teeth are prepared for sawing. Teeth from calves and yearlings are identified based on the presence of sharp occulsal surfaces, small size, thin root walls, and open apexes. These teeth are noted and removed from processing. The remaining teeth are clamped in a vice and a thin sectioning lapidary saw is used to remove the lower 10 mm of the root. The severed 10 mm root portions are placed in individual, pre-marked embedding

bags. The upper root and crown are returned to the original envelope. Teeth from deer, bear and bobcat are placed whole in pre-marked embedding bags. The root end is not cut off for these species.



**BIG GAME HUNTERS!**

Your help is needed! Personnel limitations mean trained people cannot be assigned to most check stations, or check stations are not always available, but you can assist the Game and Fish Department obtain more information on the age of the animals taken in some units. This information is valuable in the management of our animal herds. You can help by removing the two front teeth (incisors) from your animal, placing them in this envelope and turning them in at the check station. These teeth show growth rings when a section is cut away, much like those on the scales of fish. Directions for removal are included on the flip side of this envelope. Teeth can be pulled easier shortly after the animal is dead. The procedure will take only a few minutes of your time, but your assistance can aid our management program. Your cooperation will be greatly appreciated.

DIRECTIONS

As soon as possible after taking your big game animal, please collect the two front teeth (see drawing) following these steps:

1. With your hunting knife, cut down through the gum line on each side of the two teeth. CAUTION — do not break the crowns off at the gum line.
2. Move the teeth back and forth (some pressure is needed) until they can be cut free.
3. Enclose the two whole teeth (crown and root) in the envelope, provide the following information and turn in at check station or nearest Game and Fish office.

Date: _____

Name: _____

Address: _____

Species: _____ Sex: ☐ M ☐ F

Hunt Area: _____ Drainage: _____

License Number: _____




Fig. 9. Tooth collection envelope, instructions to hunter and directions for pulling teeth from ungulates.

Bagged teeth are immersed in a decalcification solution (buffered HCl) with stirring, until they are softened (24-72 hours depending on species.) Afterward, teeth are rinsed 24 hours in tap water, and then removed from the embedding bags. Teeth from elk, moose and bison are halved lengthwise with a sharp scalpel, placed in a pre-marked, embedding cassettes and set temporarily back in tap water. Cassettes with teeth are then loaded in a VIP® tissue processor and

passed through a series of alcohol, Pro-Par® clearing agent, and paraffin baths to obtain paraffin infiltration. This is followed by treatment in a vacuum infiltrator and finally, the root portion is mounted in base molds and embedded in paraffin using the Tissue Tek II® embedder. After the paraffin block containing the tooth is hardened, the block is ready for sectioning. A Leica® bench top microtome is used to face the paraffin tooth block and cut several sections 8-10 µm thick. The sections are then floated onto a labeled slide. These are permitted to dry overnight on a warming plate. Paraffin residues are removed from the slides through a second series of alcohol and Histo-solve® baths. The slides are dried and stained in Giemsa® staining solution.

Teeth from mule deer, white-tailed deer, and bobcat are sectioned in a frozen state, after decalcification. This procedure is faster but sacrifices some cytological detail. A Reichert-Jung Cryocut® microtome kept at (-) 18º C is utilized. Three to four sections, 8-10 µm thick are mounted on labeled slides, allowed to dry overnight and stained using Giemsa® staining solution. Stained slides are dried overnight before cover slipping. Each slide is examined under a compound microscope at 40X or greater magnification. Cementum annuli are counted and the number is marked on the slide.

The numbers of annuli recorded on each slide, calculated ages, and the ages of the calf and yearling teeth obtained by inspection are recorded in a computer database established for each species. Age estimation for various species, based on cementum annuli, follows the models developed and published by Matson (1981). Age reports are forwarded in hard copy or electronic form, to wildlife coordinators and regional biologists. A computer-generated post card is also sent to notify hunters, who submitted complete address information, of the age of their harvested animal.

Consult Vieyra et al. (2004) in the Wyoming Game and Fish Laboratory Tooth Aging Procedures Manual for additional details regarding tooth analysis procedures. This document is available from the Laboratory upon request.

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