Game Techniques Manual

BIG GAME AUTOPSY SUPPLIES
April, 1971

Charles V. Lucier

Quantities and kinds of supplies needed are in part dependent on numbers and kinds of animals, numbers of workers, feasibility of resupply and other variables. Minimum essential items are capitalized.

Cutting implements

KNIVES
WHETSTONE
SAW
scalpel handles and blades
sharpening steel
axe

Equipment and miscellaneous items

CENTRIFUGE and CENTRIFUGE TUBES
LIGHTER
SYRINGES, 5 ml, 10 ml, 50 ml
HYPODERMIC NEEDLES, 18G and 13G x 3 1/2"
STERILE SWABS and TRANSPORT MEDIA
TAPE MEASURE
SCALE OF APPROPRIATE CAPACITY
MATCHES
glass slides
pressurized slide fixative
vacutainer tube holder
microscope and lamp
forceps
stop watch
innoculating wire loops
tackle box for misc. items
alcohol burner
scissors
teasers

Containers

GARBAGE CAN(S)
BUCKET
dishpan

Preservatives and fuel

ALCOHOL, 70%
FORMALDEHYDE or FORMALIN SOLN.
AFA soln.
alcohol, 95% for fuel

Personal equipment

RUBBER GLOVES
FIRST AID SUPPLIES
aprons
drug antagonists

Labeling and packaging supplies, records

LABELS (white plastic)
STRING, CORD
PENCILS (soft lead)
BAGS, PLASTIC, small, med., large
BAGS, WHIRL-PAK
MASKING TAPE, 3/4" width
FIBER TAPE
CLIPBOARD
AUTOPSY FORMS
FORMS as required
PAPER TABLET
VACUTAINERS, 5 ml and larger
VIALS, GLASS, 1" x 4" with screw caps
VIALS, PLASTIC, 3 oz. with screw caps
PAPER TOWELS
ball point pen
label wires
felt marker
cheesecloth
rubber bands

TOOTH CEMENTUM AGING TECHNIQUE FOR MOOSE

June, 1971

Robert Rausch and Carl McIlroy
Drawings by Dave Harkness

Acquisition

Properly prepared and preserved moose teeth are essential for good results. The following procedures for collection, preparation, and preservation of moose teeth should be followed. Collect both I_1 teeth preferably, although any two incisors or canines are usable. The tooth root should not be broken. Trim off all tissue adhering to the tooth roots. Place both teeth in a 20 ml screw-top, glass vial that is half filled with 2 percent formalin (1 percent formaldehyde). Include a white plastic tag, labeled with a pencil, showing the specimen number and date of kill (or date of tooth extraction if taken from a living moose). Tighten the screwcap firmly to prevent loss of fluid. Seal the screwcap with plastic electricians tape if prolonged storage is anticipated.

Grinding

Soak the teeth in water overnight if they have dried through leakage from the vial while in storage. The water-bath grinder causes less odor, prevents burning of the teeth, and is self-cleaning to a greater degree than a grinder without a water-bath. Hold the tooth with the lingual (tongue) side up and use a rubber stopper to press the lower 2/3 of the root on to the abrasive wheel. Grind away the lateral surface. Check frequently and stop grinding when the pulp canal becomes uniformly exposed. Reverse the tooth, lingual side up, and grind away the opposite lateral surface. Continue grinding alternately on opposite sides of the tooth root until the remaining section is median and is becoming translucent. Check the tooth root occasionally using a binocular dissecting microscope at 20x to 30x magnification and cease grinding when the cementum annuli become distinct. Wash the grinding debris off the tooth and blot the tooth root dry. The tooth should now resemble Figure 1.

Age Determination by Counts of Cementum Annuli

The following age determination procedure should be regarded as provisional until known age moose teeth become available. Use a binocular dissecting microscope at 20x to 30x magnification to examine the tooth root. The cementum will appear as a series of alternating translucent bands and opaque lines. By definition, translucent bands will be called summer bands and opaque lines will be called winter lines. Starting at the dentine-cementum interface, the first opaque line lies close to the dentine and corresponds to the first winter of life. The innermost, broad, translucent band corresponds to the second summer of life. The second opaque line corresponds to the second winter of life. Especially on older animals, however, the second summer band is opaque, and the first winter, second summer, and second winter of life are represented by one broad, opaque band.

For aging purposes, therefore, this broad, opaque band represents 2 years of life. Count all opaque lines to the outer border of the cementum to derive the age. A moose killed in early fall will have a terminal summer (translucent) band. Moose killed in late fall may have a terminal winter (opaque) line, but this terminal line should not be counted in fall-killed moose. Moose killed in late winter or spring, but before an arbitrary birth date of June 1, may have a terminal translucent band. The moose's age in this case is the number of opaque bands minus one. Secondary lines and splitting of lines are occasionally seen and may be confusing, but they can usually be resolved by observing cementum annuli over broad areas of the tooth root margin. The cementum of a moose tooth assumed to be 7 years old is diagrammed in Figure 2, and it illustrates many of the features discussed.

Figure 1. Diagram of a moose tooth illustrating the surfaces to be ground away for age determination by cementum layering.

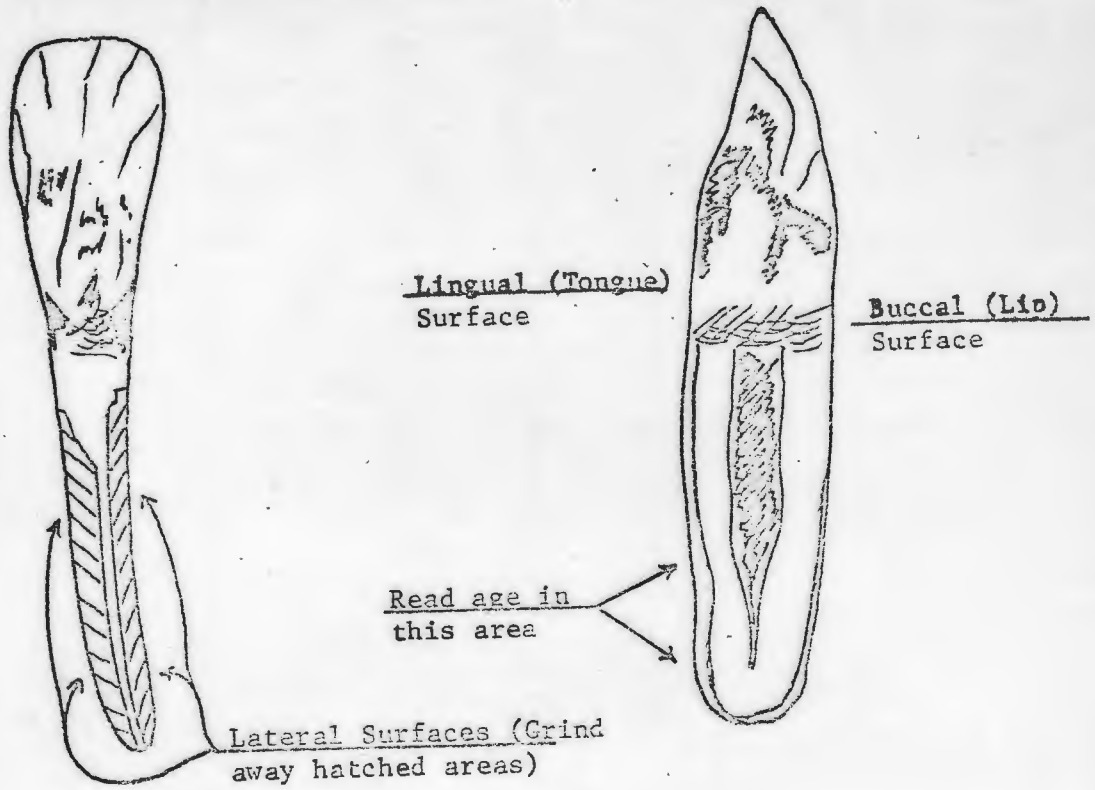
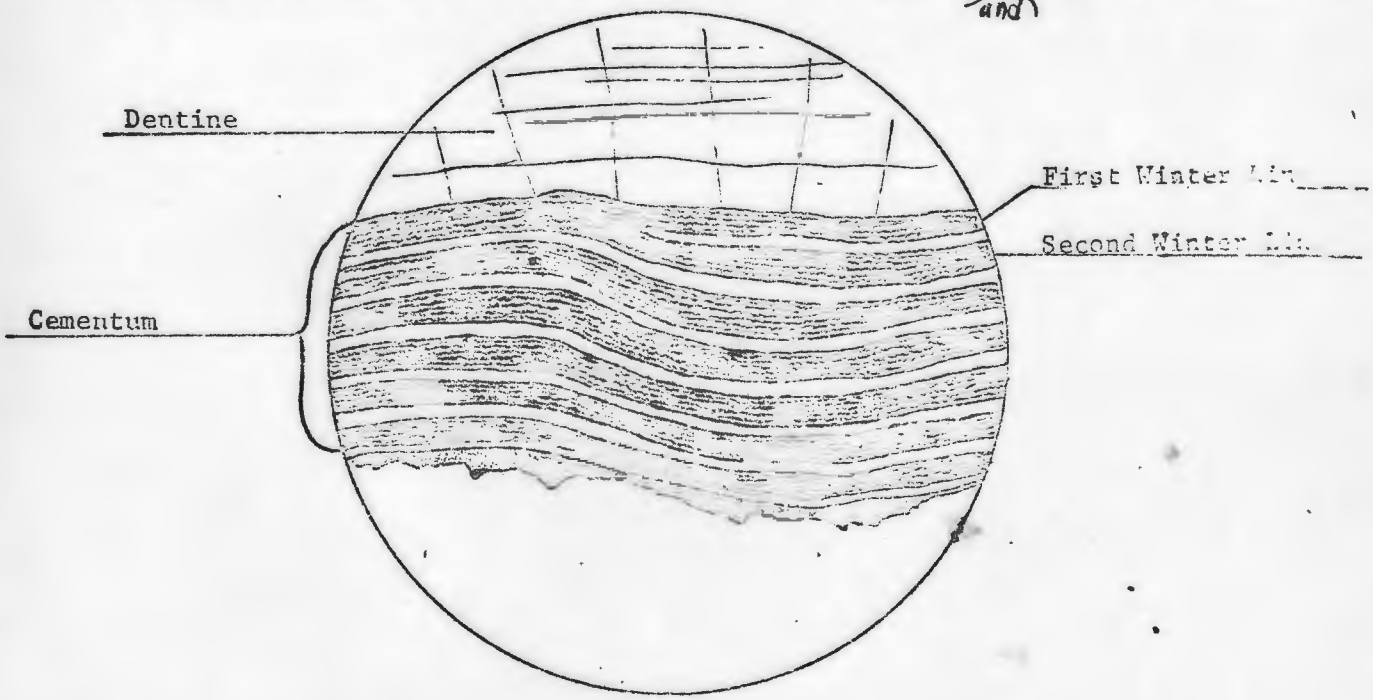


Figure 2. Diagram of a moose tooth section showing cementum layering under 60X magnification. The tooth specimen is from a moose killed in early fall and is aged at 7 years.



PREPARATION OF POLAR BEAR TEETH FOR AGING BY COUNTING CEMENTUM ANNULI

June, 1971

Nick Steen and Carl McIlroy

Acquisition

Hunters are required to present polar bear hides and skulls to Alaska Department of Fish and Game personnel for sealing. An M_3 or PM_3 is removed at this time. The teeth are forwarded to the Anchorage office for processing, and they arrive either in vials containing preservative or in envelopes. Teeth arriving in envelopes should be placed in vials of preservative to prevent shattering from excessive dryness.

Decalcifying

Place the tooth and label in a 3 oz. plastic, screw-top vial containing 2 oz. of decalcifying solution (Decalcifying Solution, Cat. No. D1208, Scientific Products, Evanston, Illinois). Premolars require 1 to 2 days to decalcify; molars require 4 to 7 days. The tooth will be flexible when it is decalcified. Any remaining calcified areas within the tooth may be detected by piercing the tooth with a dissection probe. Allowing the tooth to remain in the decalcifying solution after decalcification is complete may cause sloughing of cementum annuli.

Place each decalcified tooth along with its label in a separate space of a perforated, compartmented, plastic box. The box lid should be secured with a rubber band. Immerse the plastic box in continuously exchanging fresh water for at least 6 hours to rinse out all remaining acid.

Sectioning

Sections from polar bear teeth should be approximately 40 microns thick. Operation of the cryostat is described in a separate report.

Storage

If the tooth is decalcified but not sectioned, store it in water at 35° to 40° F for no longer than 1 week.

If the tooth is decalcified and sectioned, store all tooth sections, remains of the tooth, and the label in a screw-top, glass vial containing a preservative. The preferred preservative is 98 parts of 30 to 70 percent aqueous ethanol solution with 2 parts of glycerine added to retard evaporation.

If the tooth must be resectioned after storage in a preservative, rinse the tooth in running water for 6 hours before sectioning.

Staining

Cementum annuli in polar bear teeth stain poorly in comparison to other species. The following staining technique, however, is the best one developed to this date. Place the tooth sections in a beaker of water and select six pulp sagital sections which most nearly include the center of the pulp cavity. Float these sections onto a clean slide, arranging them so that they can be covered by a 24mm x 50mm cover glass. Place the slides in an uncovered slide box and allow them to air dry for a minimum of 6 hours.

Dilute multiple stain (Paragon Multiple Stain for Frozen Sections, PS 1301, Paragon C & C Co., Inc., Bronx, New York) with an equal volume of tap water. Fill a copeland jar three-fourths full of diluted multiple stain. Place four air-dried slides in the stain at 15 second intervals, and allow each slide to remain in stain for 1 minute. Rinse the slides in running water to remove excess stain, place them in a beaker of water until they become blue. Place the slides in a slide box to air dry overnight. Then place each slide for not less than 30 seconds in a copeland jar which contains toluene. Blot the slides dry with disposable wipers (Kimwipes^{R}, type 900-S, Kimberly Clark Corp., Neenah, Wisconsin) and cover the sections with a cover glass using Histoclad^{R} (Clay Adams, Div. of Becton, Dickinson, and Co., Parsippany, New Jersey). Allow the slides to remain in a horizontal position for one week at room temperature before storing them in a slide box.

PREPARATION OF BROWN BEAR TEETH FOR AGING BY COUNTS OF CEMENTUM ANNULI

July, 1971

Nick Steen and Carl McIlroy

Acquisition

Hunters are required to present brown bear hides and skulls to Alaska Department of Fish and Game personnel for sealing. A PM_1 or PM^1 is removed at this time. If large teeth such as molars, canines, or third incisors arrive in envelopes, they should be placed in vials of preservative to prevent their shattering due to excessive dryness.

Decalcifying

Place the tooth and label in a 3 oz. plastic screwcap vial which is half filled with a 4 percent aqueous hydrochloric acid solution. Premolars require approximately 2 days to decalcify; molars require up to 1 week. The tooth will be flexible when it is decalcified. Any remaining calcified areas within the tooth may be detected by piercing the tooth with a dissection probe. Allowing the tooth to remain in acid after the tooth is completely decalcified may result in a loss of cementum annuli.

Place each decalcified tooth along with its label in a separate space of a perforated, plastic, compartmented box. The box lid should be secured by a rubber band. Immerse the plastic box in continuously exchanging fresh water for at least 6 hours to rinse out all remaining acid.

Sectioning

Sections from brown bear teeth should be approximately 40 microns thick. Operation of the cryostat is described in a separate report.

Storage

If the tooth is decalcified but not sectioned, store it in water at 35° to 40° F for no longer than 1 week.

If the tooth is decalcified and sectioned, store all tooth sections, remains of the tooth, and labels in screwtop glass vials containing a preservative for an indefinite time limit. The preferred preservative is 98 parts of 30% to 70% aqueous ethanol solution with 2 parts of glycerine added to retard evaporation.

If the tooth must be resectioned after storage in a preservative, rinse the tooth in running water for 6 hours before sectioning.

Staining

Place the tooth sections in a dish of water and select six sagital sections which most nearly include the center of the pulp cavity. Transfer

these sections a a petri dish of undiluted hematoxlyn (Harris Hematoxylin modified for Papanicolaou staining, PS1291, Paragon C. & C. Co., Inc.) for approximately 2 minutes. Rinse the tooth sections in water and transfer them to a vial of water until they develop a blue coloration. Dehydrate the sections in successive 2-minute baths of 70%, 95% and 99% aqueous ethanol and immerse them in toluene for approximately 10 seconds. Keep the sections flat during their immersion in 70% ethanol by sandwiching them between perforated, stainless steel plates. Blot the sections on disposable wipers (Kimwipes®, type 900-S, Kimberly Clark Corp., Neenah, Wisconsin) after each immersion in alcohol to reduce contamination of the subsequent baths. Place the sections on a labeled slide and carefully cover the sections with a coverslip using Histoclad® (Adams Histoclad®, No. A-1399, Clay-Adams, Inc.) as a mounting media. Allow the slides to dry in a horizontal position for several days before storing them in a slide box.

PREPARATION OF SEA OTTER TEETH FOR AGING BY COUNTS OF CEMENTUM ANNULI

July, 1971

Karl Schneider, Carl McIlroy and Nick Steen

Acquisition

Sea otter are usually obtained from scientific collections or from accidental deaths during transplant projects. Sea otter skulls are forwarded to the Anchorage office with the teeth in situ. The responsible biologist should be consulted before tooth extraction or skull cleaning to find out if special procedures are needed. The standard processing procedure is described below.

The PM_1, I^3, and PM^1 are listed in order of preference for age determinations. Select two teeth and remove them by chipping bone away from the tooth root (the bulbous root tip prevents direct tooth extraction). Allow the skull to decompose at room temperature for one week to facilitate removal of teeth. Storage of teeth is discussed in a subsequent section.

Decalcifying

Place a label and one of the two teeth from each sea otter in a 3 oz. plastic, screw-top vial containing 2 oz. of 3 percent aqueous hydrochloric acid. The tooth will require 1 to 2 days to decalcify and will become flexible. Any remaining calcified areas within the tooth may be detected by piercing the tooth with a dissection probe. Allowing the tooth to remain in acid after decalcification is complete may cause sloughing of cementum annuli.

Place each decalcified tooth along with its labeling in a separate space of a perforated, compartmented, plastic box. The box lid should be secured with a rubber band. Immerse the plastic box in continuously exchanging fresh water for at least 6 hours to rinse out all remaining acid.

Sectioning

Frozen sections from sea otter teeth should be approximately 34 microns thick. Operation of the freezer-microtome (cryostat) is described in a separate report.

Storage

If the tooth is decalcified but not sectioned, store it in water at 35° to 40°F for no longer than one week.

If the tooth is decalcified and sectioned, store all tooth sections, remains of the tooth, and the label in a screw-top, glass vial containing a preservative. The preferred preservative is 98 parts of 30 to 70 percent aqueous ethanol solution with 2 parts of glycerine added to retard evaporation. Do not use formaldehyde as a preservative. Formaldehyde causes the outer layers of cementum to turn black and prevents accurate age determinations.

If the tooth must be resectioned after storage in a preservative, rinse the tooth in running water for six hours before sectioning.

Staining

Place the tooth sections in a dish of water and select six sagital sections i.e., in the median longitudinal plain which most nearly include the center of the pulp cavity. Transfer these sections to a petri dish containing a working solution of Giemsa stain. A working solution of Giemsa stain is prepared by adding 1.0 ml of Giemsa Buffer Solution (Giemsa Buffer Solution, PS 1165, Paragon C. & C. Co., Inc., Bronx, N.Y.) and 0.5 ml of Giemsa Stock Stain Solution (Giemsa Stain, PS 1161, Paragon C. & C. Co., Inc., Bronx, N.Y.) to 23.5 ml of distilled water. Staining requires 10 to 12 minutes. Briefly immerse a tooth section in a beaker of water and float it onto a labeled slide. Float five more sections from the same tooth onto the slide. Then place the slide in a slide box to air-dry for 16 to 18 hours.

Giemsa stain is water soluble, so exposure to water should be of short duration. The working solution of Giemsa stain is disposed of upon formation of a precipitate. Precipitate will usually form after approximately 30 minutes of use.

Mounting

Place the air-dried slides in a copeland jar containing toluene for at least 30 seconds. Remove the slides and blot them dry with disposable wipers (Kimwipes®, type 900-S, Kimberly Clark Corp., Neenah, Wisconsin). Cover the sections with a coverslip using Histoclad® (Clay Adams, Division of Becton, Dickinson, and Co., Parsippany, New Jersey) as a mounting media. Allow the slides to dry in a horizontal position for one week before stacking them vertically in a slide box.

OPERATION OF THE CRYOSTAT

July, 1971

Nick Steen and Carl McIlroy

Specimen Preparation and Operation

Refer to the appropriate species in the Wildlife Specimen Techniques Manual for details on tooth decalcification and rinsing procedures. Insufficient decalcification or rinsing may result in damage to the microtome knife edge. The operator should read the instruction manual and understand the nomenclature and function of the cryostat's components before attempting any operations.

Mounting Incisors and Single-Rooted Premolars

Engage the quick-freeze unit for 3 minutes to completely chill the specimen holder plates. Place successive drops of water on each of the plates, allowing each drop of water to freeze and cumulatively form a mound of ice. This mound of ice will support the tooth above the specimen holder plate and reduce the possibility of damage to the microtome knife edge. Position the tooth on the mound of ice so that the tooth's labial or lingual surface is parallel to the surface of the specimen holder plate. Cover all portions of the tooth with ice by allowing successive drops of water to freeze on the tooth and supporting mound of ice.

Mounting Molars and Double-Rooted Teeth

Engage the quick-freeze unit for 3 minutes to completely chill the specimen holder plates. Place successive drops of water on each of the plates, allowing each drop of water to freeze and cumulatively form a mound of ice. Bisect a tooth with a razor blade along a median plane perpendicular to the tooth's lingual or labial surface. Place one of the halves of a bisected tooth on the specimen holder with the cut surface parallel to but facing away from the specimen holder plate. Cover all portions of the tooth with ice by allowing successive drops of water to freeze on the tooth and supporting mound of ice.

Sectioning Teeth

The cryostat should have an oversized index post (Part No. 3347) installed. The following instructions apply when sections of 32-46 microns thickness are desired. Adjust the index setting to produce sections of the desired thickness. Place a plate and mounted tooth in the specimen holder. Position the specimen holder in the slide block so that the tooth will be sectioned along its longitudinal axis, from root tip to crown, to produce median sections. The best sections are those showing the entire pulp cavity. Since intact teeth are conical, tapering from crown to root apex, initial sectioning should remove material from the crown of the tooth so that subsequent successive sections will be parallel to the

pulp cavity. The tooth should be properly positioned before the pulp cavity is exposed to avoid loss of the best sectioning area. Discard sections until the pulp canal appears, then collect 12 subsequent sections. Collect sections from molars and double-rooted teeth after initial sections have produced a smooth, even surface from the bisected face of the half tooth. Place the sections, remains of the tooth, and a label in a screw-top glass vial containing 98 parts of 30 to 70 percent aqueous ethanol solution with two parts of glycerine added to retard evaporation.

The following special instructions apply when sections of 64-92 microns thickness are desired. Adjust the index setting for one half of the desired section thickness. Bring the slide block to its starting position (i.e., an uppermost position farthest from the microtome knife) by rotating the microtome hand wheel. The specimen holder and mounted tooth should be positioned in the slide block as has been described in the preceding paragraph. Slowly rotate the microtome hand wheel until the pawl on the rachet wheel reaches the end of its travel (this is indicated by a cessation of audible clicks and occurs approximately within a 45° arc of a complete 360° hand wheel rotation). Then reverse the microtome hand wheel rotational direction to return to its starting position. Again reverse direction and bring the microtome hand wheel through a complete 360° rotation. This maneuver will produce one section twice as thick as indicated by the index setting on the index post. Collection and storage of sections are described in the preceding paragraph.

Precautions

1. Frost accumulates relatively rapidly on the quick-freeze unit and on the cooler walls and reduces the cryostat's cooling efficiency. Frequent defrosting is necessary.

2. Discarded sections on bearing surfaces cause wear and inaccurate sectioning. Bearing surfaces should be kept clean.

3. The operator's hands should be dry when adjusting the ratchet wheel. Moisture from wet hands may collect on the ratchet teeth and cause skipping with consequent thick sections.

4. The operating temperature of the cryostat is maintained at -15° C. Contact between metal parts and skin may cause frost burns.

5. Remove the microtome knife before cleaning the cold chamber to avoid accidental injury.

OPERATION OF THE THIN SECTIONING MACHINE

July, 1971

Nick Steen, Charles Lucier, and Carl McIlroy

Acquisition

The teeth of caribou, deer, elk, bison, sheep, and goat and occasionally other species are processed on the thin sectioning machine. Teeth from these species may arrive either dried or in a preservative. Tissue adhering to the teeth need not be removed before embedding.

Embedding

Place two layers of overlapping paper towels in a shallow 18 x 24 in. tray, print the accession numbers of the teeth to be embedded in vertical columns on the towels with 1 inch vertical spacing between the numbers, and 2 inch horizontal spacing between the columns, and position each tooth to the right of its corresponding number. Place a strip of masking tape over each column of teeth, attaching the teeth in place to the towels. Place the tray under the fume hood overnight to air-dry the teeth. Record the teeth accession numbers in three columns with 1 inch vertical spacing between numbers and 2 inch horizontal spacing between columns on a sheet of 8 1/2 x 14 in. lined paper. Pour a half inch deep layer of Bio-plastic into an 8 x 14 in. glass baking dish, place the paper bearing teeth accession numbers under the baking dish, and immerse the teeth in the uncatalyzed Bio-plastic overnight, each tooth overlying its corresponding accession number. Bio-plastic should be covered and kept under the fume hood, making certain that the blower is turned on, to reduce the hazard of vapor toxicity.

Arrange the embedding molds (Peel-A-Way® Cup, Peel-A-Way® Scientific, South El Monte, Calif.) in columns, each embedding mold positioned with its two internal protuberances on the right side of the column. Fill each embedding mold with Bio-plastic to the level of the internal protuberances, add three drops of catalyst (methyl ethyl ketone peroxide) to the Bio-plastic, and stir the catalyst into the Bio-plastic while avoiding induction of air bubbles into the catalyzed Bio-plastic. Remove a tooth from the baking dish containing uncatalyzed Bio-plastic, blot off excess Bio-plastic, and place the tooth in an embedding mold with the apex of the tooth root pointing toward the technician and almost touching the wall of the cup. Place a label bearing the accession number within the embedding mold and above the crown of the tooth.

A maximum of four teeth can be embedded in one mold. To embed several teeth in one mold, first arrange the embedding molds in columns with the two internal protuberances of each mold on the right side of each column. Then arrange the teeth so that the second quarter of all tooth roots, numbered sequentially starting at each root apex, form a straight line with the teeth roots pointing toward the technician. Maintain uniform spacing between the teeth but allow a 1/4 inch space on the right side of the embedding mold. Each accesion number on the label should be written directly above the crown of the corresponding tooth.

Allow the embedding molds to remain undisturbed at room temperature overnight, and then place them in a warm (140 to 160° F) oven overnight. Peel the molding cups away from the hardened blocks of Bio-plastic. The Bio-plastic blocks are now ready to section.

Sectioning and Dehydrating

Read the instruction manual supplied with the thin sectioning machine before attempting any operations. Remove all discarded sections left in the machine, clean the drain elbow, and blow out the drain hose. A small piece of window screening placed over the drain hole will reduce clogging of the drain tube with plastic thin section fragments. Turn the water on and adjust it for maximum flow without spraying the surrounding area. Check to ensure that both outlets are supplying water to the saw blade.

Position the block of hardened Bio-plastic in the thin sectioning machine's vise so that the label is upright and between the jaws. Allow three fourths of the block to extend beyond the vise and firmly clamp the block in this position. Adjust the position of the vise to bring the second quarter of the tooth root, counting sequentially from the root apex, directly in front of the rotary saw blade. Saw through the block and dispose of the piece of block containing the root tip. Then adjust the automatic stop to turn the machine motors off shortly before it completely severs through the block. Set the micrometer dial on zero, and return the vise table to its starting position. With the Bio-plastic block clear of the blade, move the vise table to the operator's left until the micrometer dial indicates 0.435 mm. Saw into the block to produce the first tooth section. Return the vise table to its starting position, break off the tooth section at its junction with the block, and place the tooth section in a metal tissue capsule (Tissue Capsule, Expandable, Scientific Products, Evanston, Ill.). Cut and remove three to five additional sections. A number is etched on the lid of the tissue capsules. Record this capsule number on a sheet of paper and list alongside it the accession numbers of the teeth that are placed in the capsule in the same order as they appear on the label. Snap the lid to the tissue capsule closed and place the tissue capsule in successive 10 minute baths of 50%, 95% and 99% aqueous ethanol solutions.

Mounting

Place the sections in toluene for no longer than 30 seconds. Toluene is necessary to remove all traces of ethanol, but it also softens the Bio-plastic. Carefully place the sections on a labeled slide so that each tooth section corresponds in sequence with its accession number to the left. The corner of the section that was broken from the Bio-plastic block usually provides a reference point for orientation. In addition, the shape of individual tooth cross sections are usually characteristic and provide an aid to the proper orientation of thin sections on the slide. Cover the sections with a 24 mm x 50 mm cover slip using Histoclad® (Adams Histoclad®, Clay-Adams, Division of Becon, Dickinson and Company, Parsippany, N.J.) as a mounting medium. Allow the slides to dry in a horizontal position for 1 week before storing them upright in a slide box.

PREPARATION OF TOOTH SPECIMENS FOR AGING BY GRINDING

October, 1971

Carl McIlroy

Acquisition

Conventional techniques that are used to prepare teeth for age determinations are inefficient relative to a combination machine-hand grinding technique when only a small number of teeth are to be processed. In addition, it is often necessary to rapidly produce tooth sections for aging, and a tooth specimen undergoes processing for a minimum of 2 days when using conventional techniques. Using the grinding process described below, however, less than 1 hour is required to prepare a tooth specimen for aging. All teeth normally processed in this laboratory are suitable for processing by grinding. The preparation of moose teeth by grinding is described under a separate subject heading.

Using the Rotary Grinder

Use a rotary grinder to remove most of the tooth root material. Hold the tooth with the lingual side up, press the tooth into the abrasive wheel, and carefully grind away the lateral surface. Grind the full length of teeth that are 2 cm or less in total length, but only grind the root of teeth that are longer than 2 cm. Inspect the tooth frequently and stop grinding as soon as the pulp canal is uniformly exposed. Reverse the tooth, lingual side up, and grind away the opposite lateral surface. Discontinue use of the rotary grinder when the tooth section is 1-2 mm thick and the pulp canal is uniformly exposed at both lateral surfaces. If the tooth is longer than 2 cm, score and break the tooth root transversely at the junction of the ground and unground surfaces and discard the enameled portion of the tooth.

Hand Grinding

Immerse two 4 1/2 x 5 1/2 in. sheets of 600 grit waterproof silicone carbide paper in water until they are thoroughly moistened. Lay one sheet with abrasive side up on a surface of a 1/4 x 4 x 5 in. glass plate and wrap the ends of the carbide paper around the edges of the glass plate. Lay the plate on a counter with the carbide paper on top. Wrap the other sheet of carbide paper around another glass plate and hold the ends of the paper against the edges of the plate. Moisten the abrasive surfaces of the carbide paper, lay the tooth specimen on the bottom plate, and grind the tooth section between the two glass plates by applying a light pressure and rotary movement to the upper plate. Inspect the tooth section frequently. If insufficient material is being removed from one surface of the tooth section, lay the tooth section on the bottom plate with the surface that needs grinding facing up. Hold the tooth section against the bottom plate with a finger and grind alternately on opposite halves of the upper surface until the amount of material removed from upper and lower surfaces is approximately equal. Apply less

pressure on the glass plates as the tooth section becomes translucent. If the tooth section cannot be found on the carbide sheets or on the glass plates during an inspection, immerse both carbide sheets and glass plates in a bowl of water. The missing section can usually be found as it settles to the bottom of the bowl. Continue grinding until the tooth section is nearly transparent and flexes easily around the finger tip (approximately 40 microns thick) if the section will subsequently be decalcified and stained. Ungulate teeth that are to be aged with the ultraviolet microscope should be thicker (approximately 75 microns).

Mount undecalcified tooth sections that are to be aged with the ultraviolet microscope on a microscopic slide as described in the Wildlife Specimen Techniques Manual under the subject heading "Operation of the Thin Sectioning Machine." Place those teeth sections that are to be decalcified and stained in a 4.2% v/v aqueous solution of hydrochloric acid until they are completely decalcified. Complete decalcification will require approximately 2 minutes and will be apparent by a change to nearly complete transparency and flexibility. Rinse the tooth in water for several minutes. Mounting and staining procedures are described in the Wildlife Specimen Techniques Manual under the subject heading, "Preparation of Brown Bear Teeth for Age Determinations."