A Method for Preparing Beluga (White Whale), *Delphinapterus leucas*, Teeth for Ageing

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A METHOD FOR PREPARING BELUGA (WHITE WHALE), *Delphinapterus leucas*, TEETH FOR AGEING

by

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This report is a guide for field and lab technicians who sample or age beluga. It discusses (1) methods for the field collection and storage of beluga jaws, and (2) the extraction, mounting, and sectioning of teeth in preparation for ageing.

Key words: beluga; white whale; Delphinapterus leucas; ageing.

RÉSUMÉ

Wainwright, K.L., and R.S. Walker. 1988. A method for preparing beluga (white whale), Delphinapterus leucas, teeth for ageing. Can. Manuscr. Rep. Fish. Aquat. Sci. 1967: iv + 15 p.

Ce rapport constitue un guide à l'intention des techniciens itinérants et des techniciens de laboratoire qui prélèvent des échantillons de tissus sur les bélugas et qui les analysent pour déterminer l'âge des sujets. On y présente, d'une part, les méthodes de collecte sur le terrain et de conservation des mâchoires de béluga et d'autre part, la façon de procéder pour extraire, monter et sectionner les dents en vue des analyses pour déterminer l'âge des sujets.

Mots-clés: béluga; baleine blanche; Delphinapterus leucas; détermination de l'âge.

INTRODUCTION

Age determination of various whale species, including the beluga (Delphinapterus leucas), using tooth layers has been documented by many researchers (8rodie 1969; Finley et al. 1982; Hohn 1980; Kasuya 1972; Lockyer et al. 1981; Marsh 1980; Scheffer and Myrick 1980; Sergeant 1959, 1973; Utrecht 1978). As a whale's tooth grows, layers of cementum and dentine are laid down within the tooth (Scheffer and Myrick 1980). One method to expose these layers is to cut a longitudinal thin section from the center of a tooth (8rodie 1969; Finley et al. 1982; Hohn 1980; Kasuya 1972; Utrecht 1978). Most authors do not provide detailed information on tooth sectioning and it is difficult to duplicate their methods. We discuss the field collection and storage of jaws, and the extraction, mounting, and sectioning of individual teeth.

DETAILED METHODOLOGY

FIELD COLLECTION AND STORAGE

To collect beluga teeth in the field, the entire jaw is removed (Figure 1). It is necessary to dissect the melon, lips, and tongue back to the lateral protrusion of the frontal or malar bone to expose the jaw. The maxilla and mandible are cut off just anterior to this protrusion. This ensures collection of even the most posterior teeth (Figure 2). The jaws must be cut smoothly and evenly, a hacksaw with a coarse blade (≤18 tpi) works well, or bone edges can puncture sample bags used for storing jaws and other tissues.

8lood and other foreign matter is rinsed from the jaws prior to freezing. When the tissues are fresh they can withstand agitation which may damage gum tissue after freezing and thawing. Jaws are frozen as quickly as possible. If tooth/gum measurements are not being taken, jaws can be fleshed with a sharp knife to reduce shipping weight and amount of tissue to be frozen. If tooth/gum measurements are to be taken the gum tissue is left intact. Identification labels are attached to the jaw, placed in the sample bag, and written on the bag. Markers are tested before use because some 'permanent' markers are removed by whale oil.

EXTRACTION OF TEETH

All work is conducted in a fume hood. Rubber gloves and a lab coat are recommended.

To simplify boiling and reduce jaw size, the mandible is separated into left and right halves by cutting through the mandibular symphysis (Figure 3) using a sharp, sturdy knife with a 15 cm blade. The semi-frozen jaw is placed teeth down on a soft wooden block to prevent damage to the teeth and the symphysis is cut through with a rocking motion of the knife. The two halves are then labelled saving the right half for boiling and returning the left half to the freezer.

For brevity, preparation of only right mandibular teeth will be discussed. Before boiling, identification tags are tied to each half jaw using approximately 30 cm lengths of cord. The cord is tied between two teeth to keep it from slipping off and is long enough so the label hangs outside the boiling pan. The flesh remaining on the jaw and adhering to the teeth contracts during boiling and may dislodge teeth. This is prevented by cutting the gum tissue to the bone with a scalpel anterior-posteriorly along each side of the tooth row and lingual-buccally between each tooth. Some teeth may be below the gum line so the cuts are extended to both ends of the jaw. Other tissue on the jaw is cut because the force of contraction may split the jaw if it was damaged during collection, dissection, or shipping.

The duration of boiling varies with jaw size, the amount of tissue on the jaw, the degree of freezing, and the number of jaws in the container. The heat source is adjusted to maintain a gentle boil. Each jaw is checked frequently to be sure that the tissue is separating from the teeth properly and any tissue that persists is cut again. If the water is at a full boil when jaws are immersed, the maximum boiling time is 20 minutes (Finley et al. 1982) because prolonged boiling of jaws may damage the teeth (Lockyer et al. 1981; Marsh 1980).

The teeth are removed using fingers to wiggle and twist them free. Firmly attached teeth are removed by sliding a blunt probe between tooth and socket to free adhering tissue. Impacted teeth, those that are twisted within the jaw, are extracted by breaking the jaw with pliers after all other teeth have been removed. If teeth are not removed immediately, the jaws are frozen or put in a water bath to prevent dessication. Dessication makes subsequent tooth extraction more difficult. Once teeth are removed, each is cleaned of remaining tissue by rubbing with paper towel or a plastic scraper. The pulp cavity of each tooth is cleaned with a probe.

Teeth are stored in individual vials labelled with specimen number, jaw quadrant (right or left, mandible or maxilla), and tooth number (Figure 4). Teeth are preserved in a 70% solution of ethanol (Marsh 1980) to prevent splitting or cracking. Replacing the moisture in the teeth with 70% ethanol improves the bond between tooth and embedding resin (Harden 1963).

The second and fifth tooth are routinely sectioned for age determination (Finley et al. 1982). Factors that may affect tooth selection are tooth straightness, degree of wear (Marsh 1980), and occlusion of the pulp cavity (Hohn 1980). We recommend preparing a minimum of two teeth per animal.

TOOTH MOUNTING

An effective method of holding a tooth for cutting is to mount it in a resin block (Marsh 1980). A mold is used to hold the resin, tooth, and label while the resin hardens. Silicone molds are ideal for resin mounting because they

will not bond to the resin, they are flexible and can be peeled from the hardened resin block, and they can be used repeatedly.

Molds are constructed using smooth blocks of polyethylene as templates. Each template is covered on five sides with a thin layer of silicone caulking. The silicone is allowed to harden. More layers are added, each layer hardening before the next is applied, until a wall thickness of 5-8 mm is built up. The silicone mold is then peeled from the template. Molds should be wide enough to hold a tooth and label and deep enough so the tooth can be covered by resin. Our molds are approximately 3 cm wide, 3 cm deep, and 16 cm long. These molds hold more than one tooth.

Labels are put in the resin with each tooth for identification (Figure 4). Labels are made from Nalgene PolyPaper Plastic Paper (Fisher Scientific No. 11-905) with numbers pencilled on both sides. Plastic paper is used because resin fluids are absorbed by normal paper which then turns transparent. Pencil is used because pen ink is dissolved by the resin fluids.

In preparation for mounting, the molds are organized in a fume hood with the teeth in their vials behind the molds. There should be enough space to allow a 5 mm gap between teeth. Teeth of similar size are selected for each mold to minimize the depth of resin required to cover them. This improves sectioning later. Small labels are lettered on both sides, checked, and set on the respective vial lids until required (Figure 4).

Polyester casting resin (J.T. Keenan Distributors) is used to mount the beluga teeth. This resin is clear so that the tooth orientation and the label remain visible. Also, this resin does not heat up excessively during polymerization and does not react with the storage alcohol. We use a resin to catalyst mix of 200:1, which jells slowly enough (20-30 minutes) so that teeth and labels can be reoriented if necessary. A fume hood is essential for embedding teeth in resin.

First, resin is mixed with catalyst and a 5 mm layer is poured in each mold (Harden 1963). Then as this layer begins to jell, the labels and towel dried teeth are arranged in the molds using tweezers. Each tooth is oriented at the appropriate angle for cutting, taking into account the curvature of each tooth. A tooth may be leaned aginst the side of the mold to maintain it in position. Once all teeth are in position, the orientation of each tooth is checked again. The first layer of resin is left to harden. Finally, more resin is mixed and added to cover the teeth. Resin blocks should not be thicker than necessary or they will be more difficult to section. The blocks are removed from the molds after 24 hours and the molds are reused; however, the blocks must cure in a fume hood for a week before continuing.

TOOTH SECTIONING

The sectioning machine we use is the Micro-matic Precision Wafering Machine (Micromech Mfg. Corp., Model No. TSM 77 (Figure 6)). This is a high speed (2250 rpm) saw with a 13 cm diameter High Concentration Diamond Wafering Blade (Buehler Ltd. No 11-4245). This machine produces 0.3 mm thick polished tooth sections. The sectioning machine is assembled and the blade is adjusted until it is just above the stage. The splash guard is put in place and the cooling and lubricating water stream is set to strike both sides of the blade evenly.

Individual tooth blocks are cut from the resin blocks containing several teeth (Figure 5a) using a jig saw. Eye protection is essential when cutting resin and teeth. Tooth blocks are cut leaving 2-3 mm of resin at the ends of each tooth block. Blocks are secured in an "Eclipse" No. 410 or 411 machinist clamp (Figure 5b) which is held onto the magnetic stage of the sectioning machine. If a tooth block is too large to be secured in a clamp, the block is trimmed into an "L" or "T" shaped block so that it can be held. Care is taken that the label remains intact.

Tooth blocks are mounted in the machinist clamp on a flat surface. The block is rested on a 1-2 mm thick spacer so that the block will be held slightly above the stage after clamping and spacer removal (Figure 7). The clamp must lie flat when it is tightened or it will not be held securely by the magnet but the tooth block may be slightly tilted in the clamp to align the best axis for sectioning. The clamp and block are placed on the cutting stage of the sectioning machine (Figure 8a). A series of parallel lines on the stage are used to align the tooth axis with the wafering blade (Figure 8b). Thick blocks are difficult to align because refraction distorts the view of the mounted tooth. Once the tooth is aligned, the magnet is activated to secure the clamp and block to the stage.

A thin section which exposes the central core of the tooth is required for age determination. Care and attention to detail is necessary to ensure that an acceptable section is produced. Safety glasses and a particle mask are needed during sectioning.

The spinning saw blade is stationary and the motor driven stage advances carrying a tooth block into the blade. The first pass of a tooth block under the blade is made to one side of center. The stage is reversed and the block is backed up free of the blade. The stage is then moved laterally the thickness of one section with the section thickness adjusting wheel. Sections of 0.3 mm are produced by moving the stage laterally 750 units. The stage is advanced into the blade again to produce a thin section. This sequence is continued until a section is made from the center of the tooth (Figure 9). It is always important to start sectioning well away from the center of the tooth or the most useful structures of the tooth for age determination may be destroyed.

Sectioning speed or the speed at which the stage advances towards the blade is controlled by a rheostat. The best sections are produced with the rheostat in the 8-9 o'clock position. The stage must be stopped by setting the rheostat counter-clockwise to 6 o'clock before flipping the reverse direction toggle or a fuse will blow. The stage can be moved quickly while reversing. Adjusting speed or thickness while the blade is cutting damages the section being cut and may break the saw blade.

The force of the spinning blade and cooling water is sufficient to throw any sections that are cut free into the slash guard. To prevent damage to the sections, the blade is not advanced all the way through the block, a small corner is left to keep the sections attached and in order (Figure 9). The remaining resin may be easily split later. Once all sections are cut from a tooth, the stage is reversed until the saw blade is clear of the block and the machine is turned off. The block is grasped so the sections cannot come apart and the clamp is freed from the magnet. Sections are now ready for storage.

A block is split to separate individual sections, and the resin surrounding each section is carefully peeled away (Figure 10). The resin is brittle and may throw shards so users should wear safety glasses. The sections are placed in their original labelled vial and covered with 70% ethanol (Marsh 1980). The residual block pieces are held together with an elastic band and stored in 70% ethanol in a larger jar.

CONCLUSION

This paper outlines one method of preparing longitudinal thin sections of beluga teeth for age determination. The sections produced by this method are 0.3-0.4 mm thick. Several factors, such as tooth size, cutting speed, and resin hardness can affect section quality. It is important to practice prior to sectioning valuable samples.

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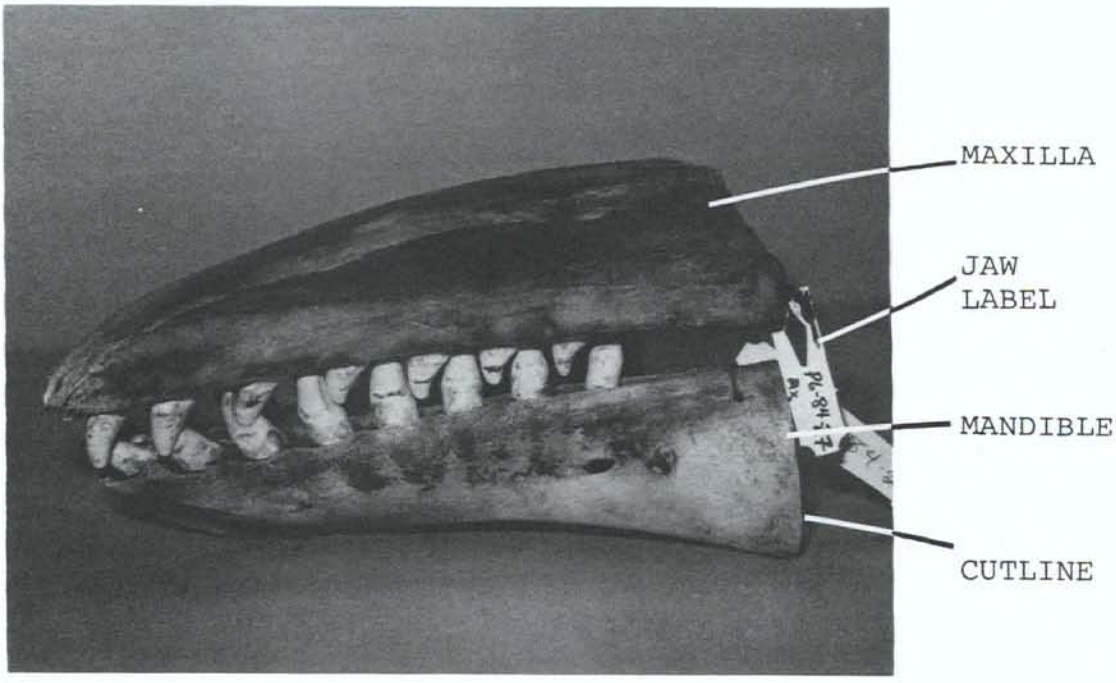
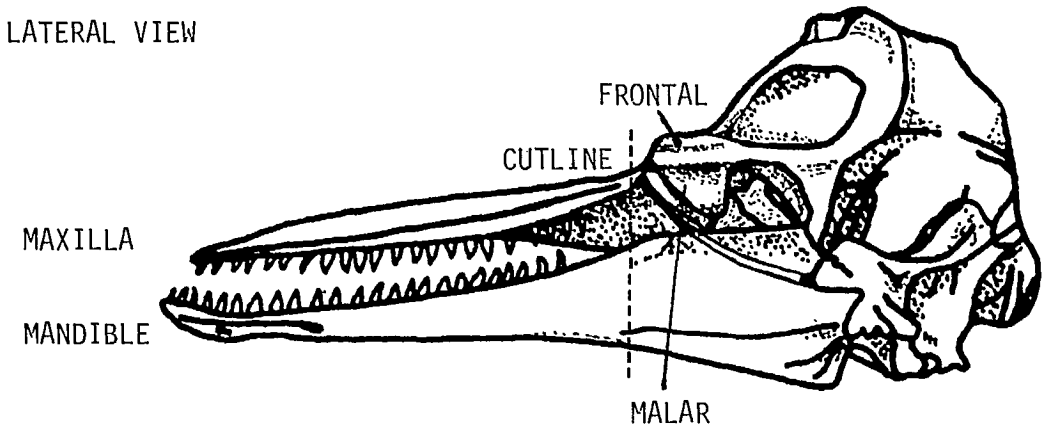
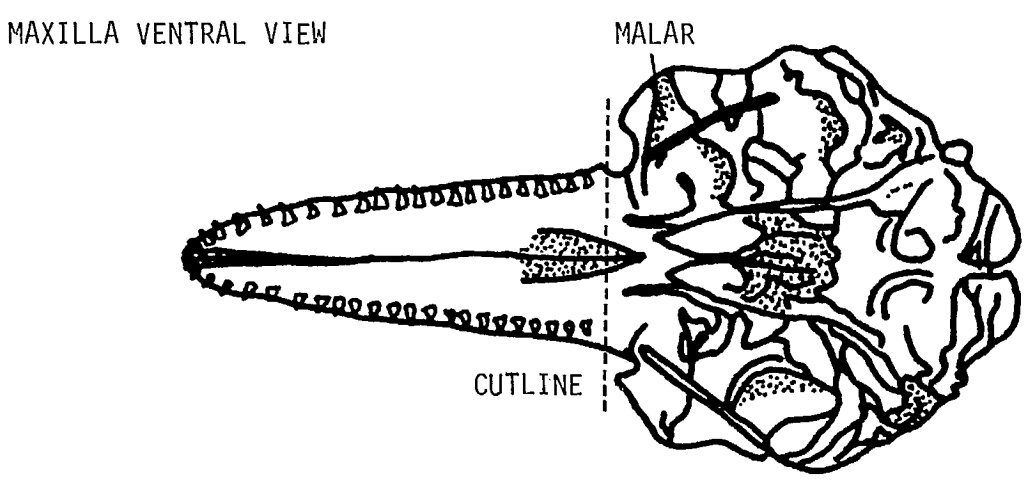


Fig. 1. Beluga jaw after removal following instructions listed in field collection and storage.





Atlantic Bottlenosed Dolphin (Tursiops truncatus)

(Green 1972)

Fig. 2. Tooth alignment within jaws showing cutlines for jaw removal.

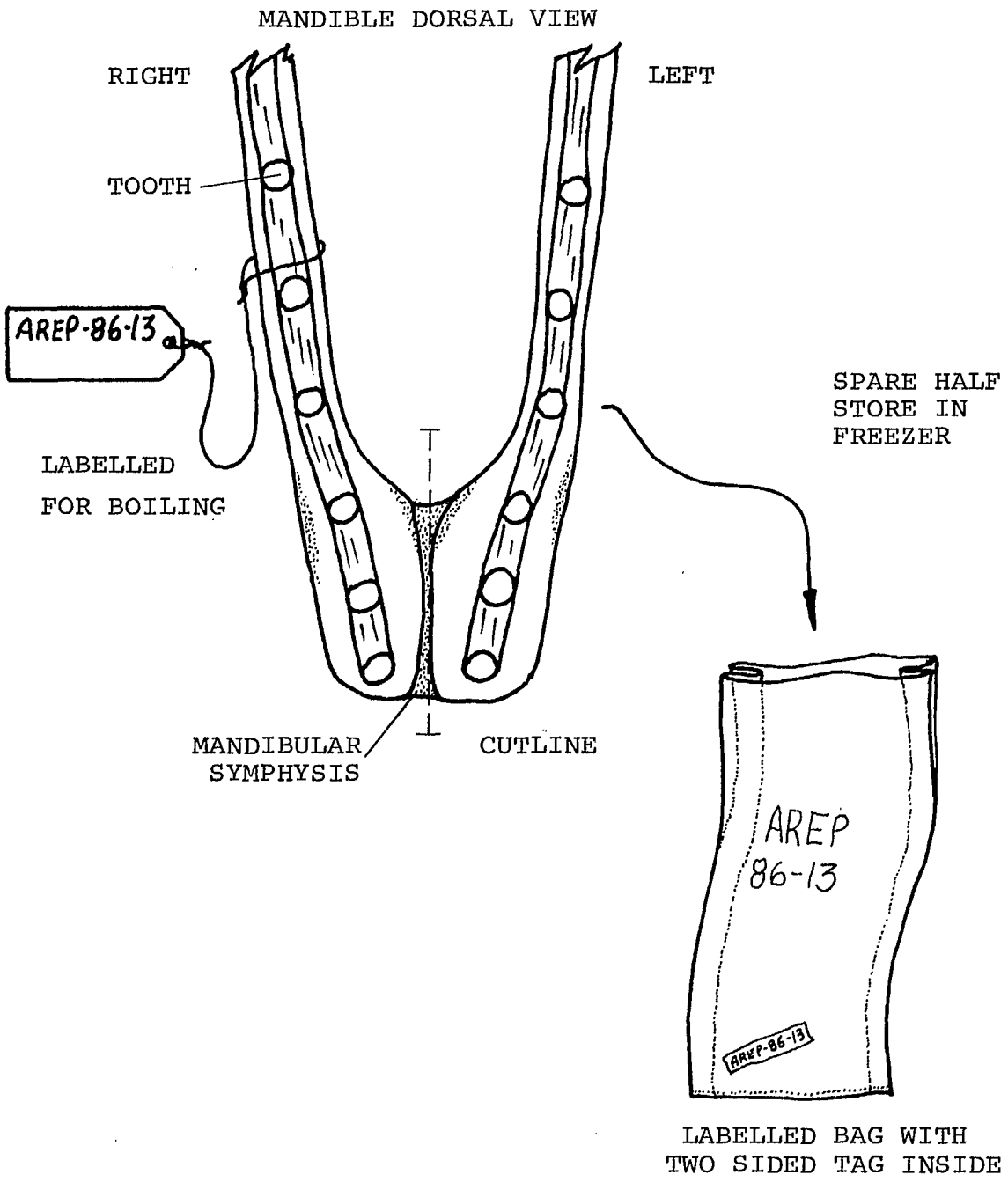


Fig. 3. Mandible bisection location and labelling.

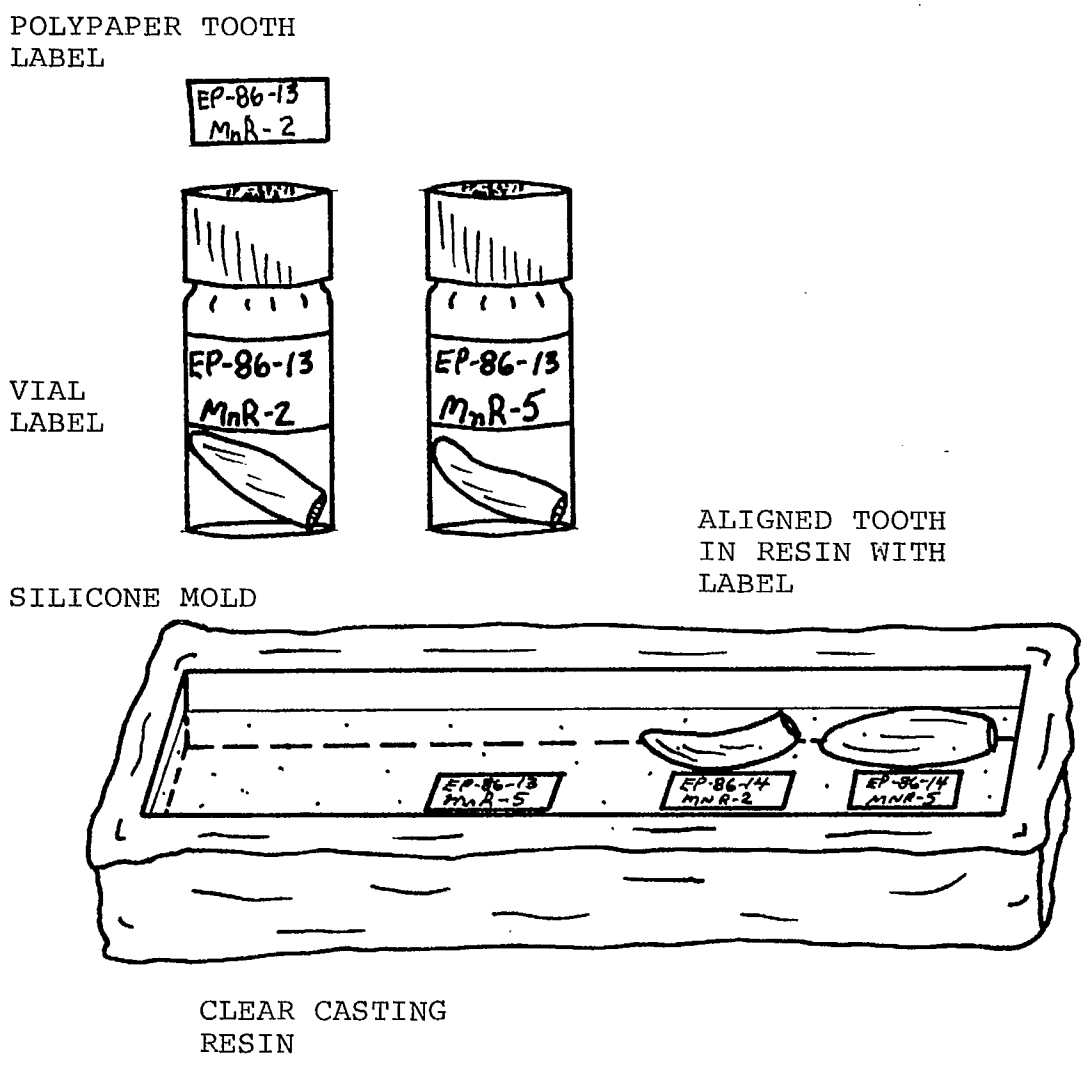
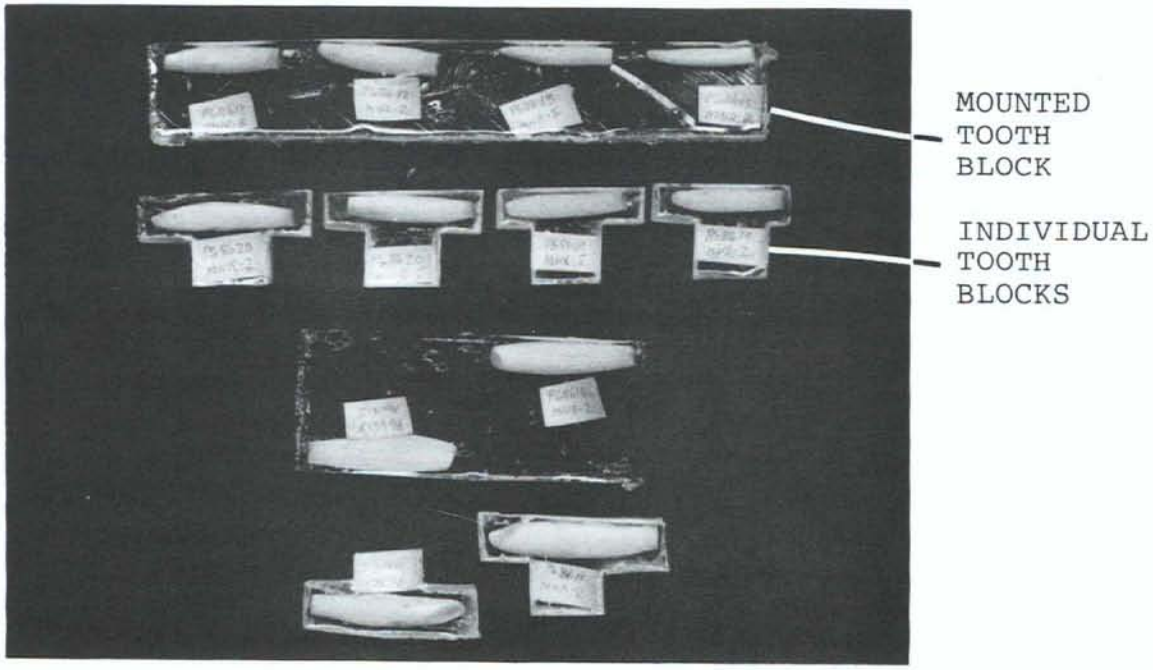


Fig. 4. Tooth mounting in mold.

A. Assorted blocks with teeth



B. Mounting individual tooth block

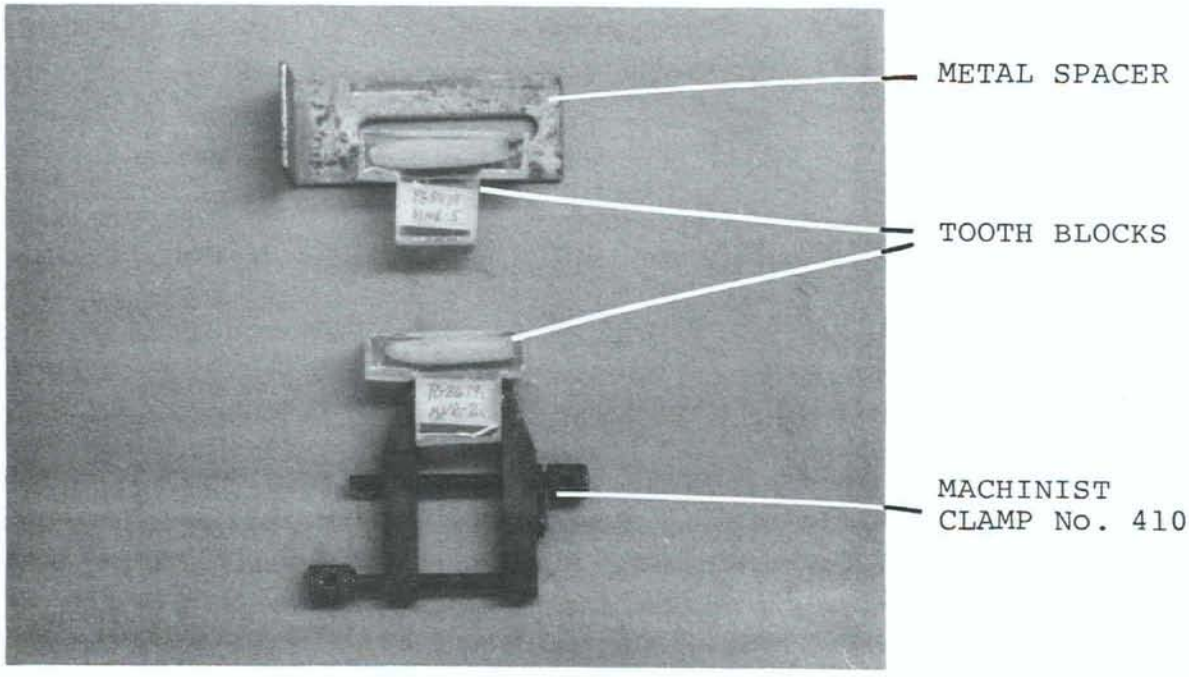


Fig. 5. Tooth block cutting and trimming.

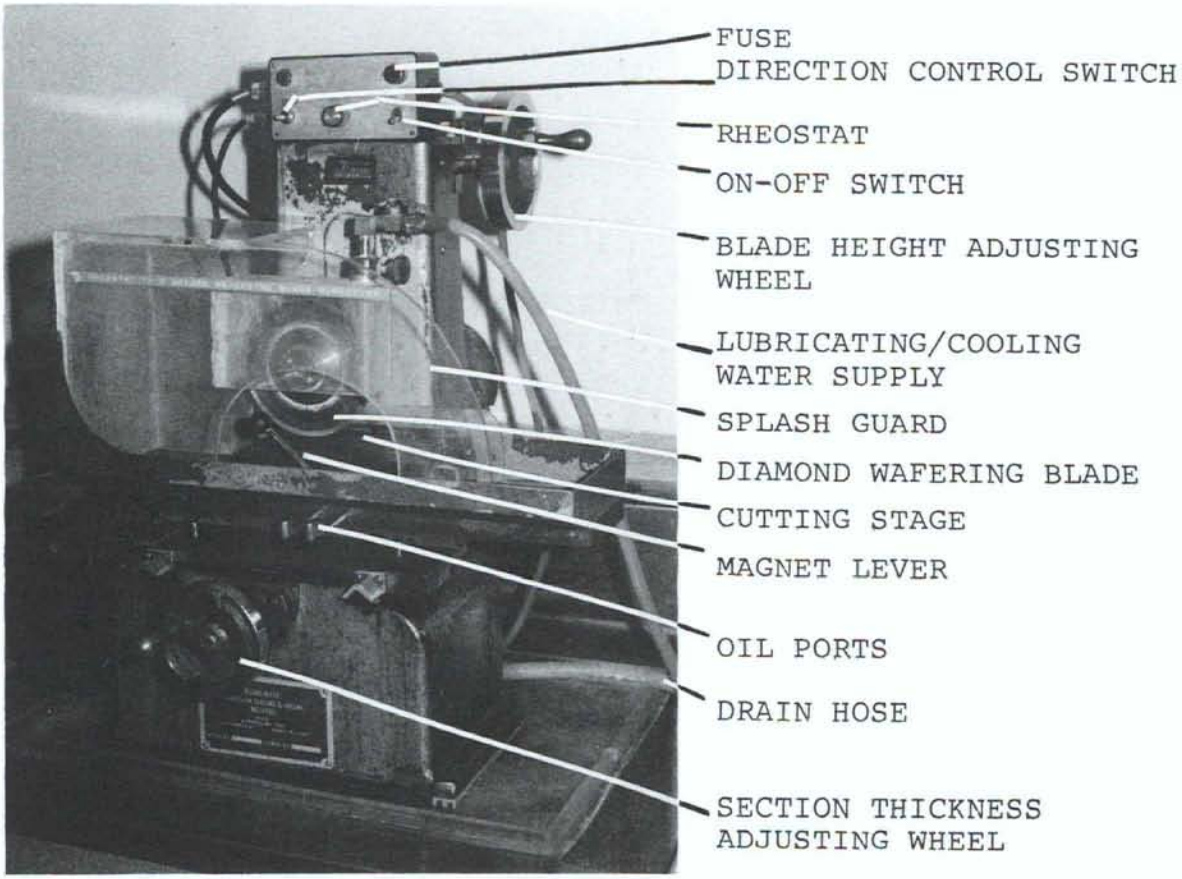
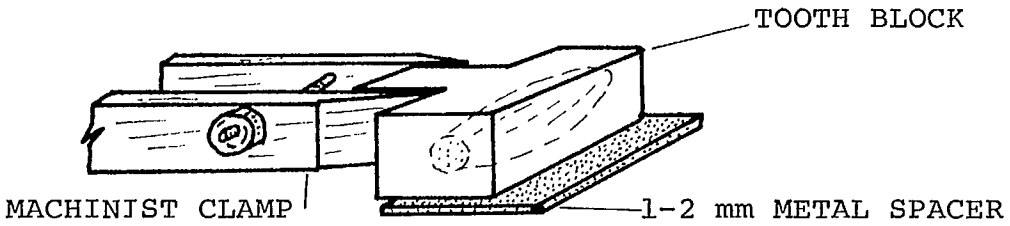
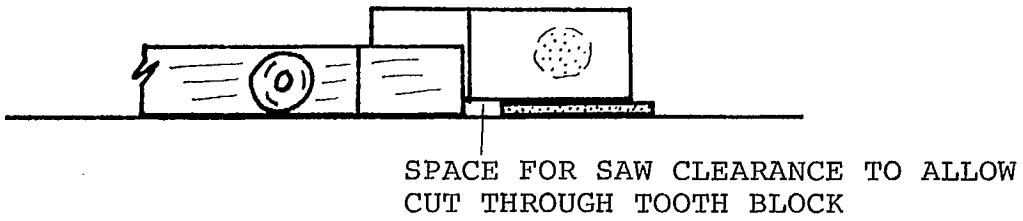


Fig. 6. Micro-matic Precision Wafering Machine, TSM 77.

A. OBLIQUE VIEW



B. SIDE VIEW



C. SAW BLADE HEIGHT ADJUSTMENT

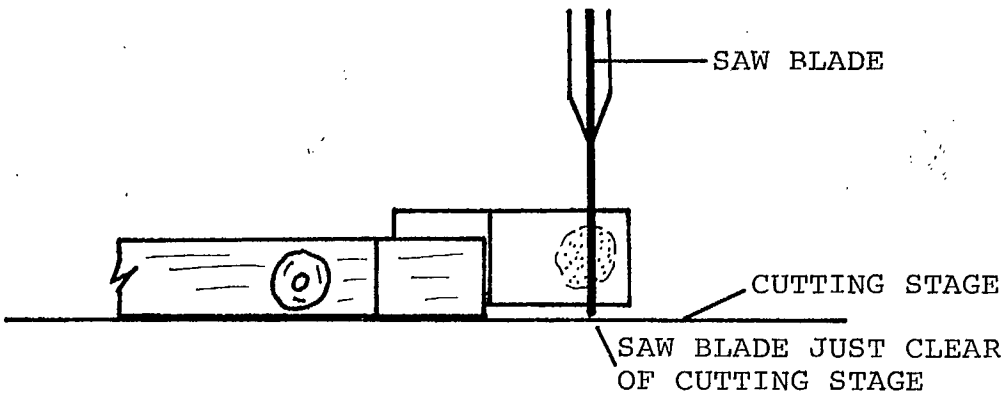
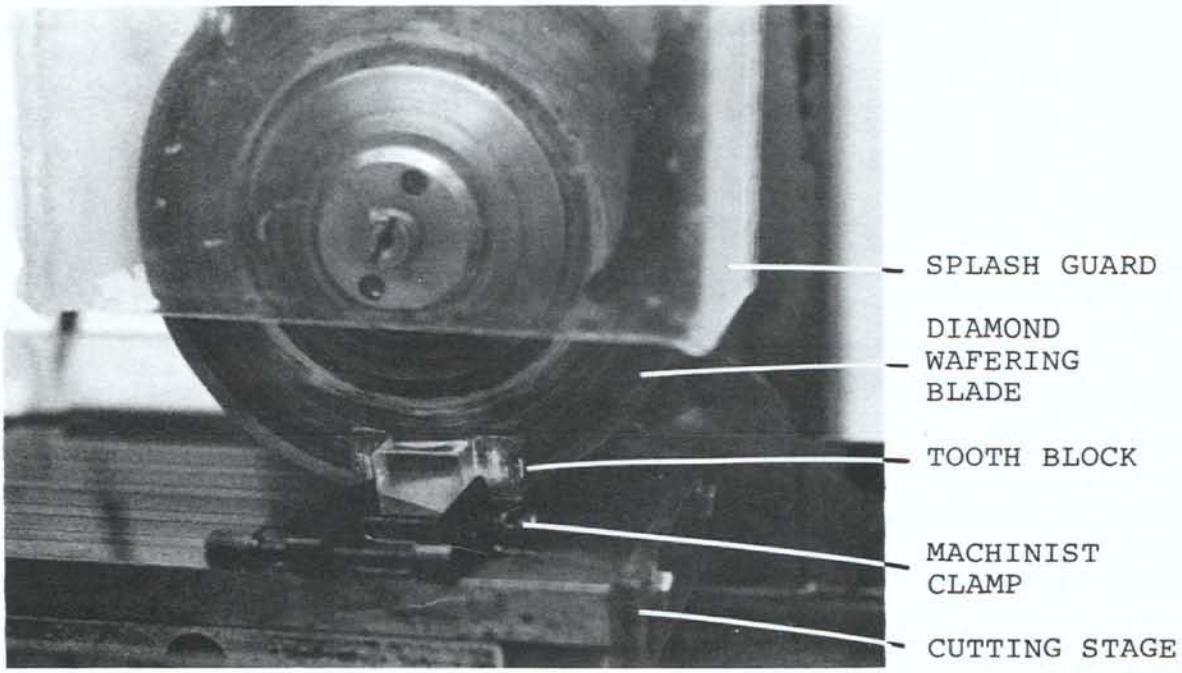


Fig. 7. Tooth block clamping.

A. Left side



B. Right side

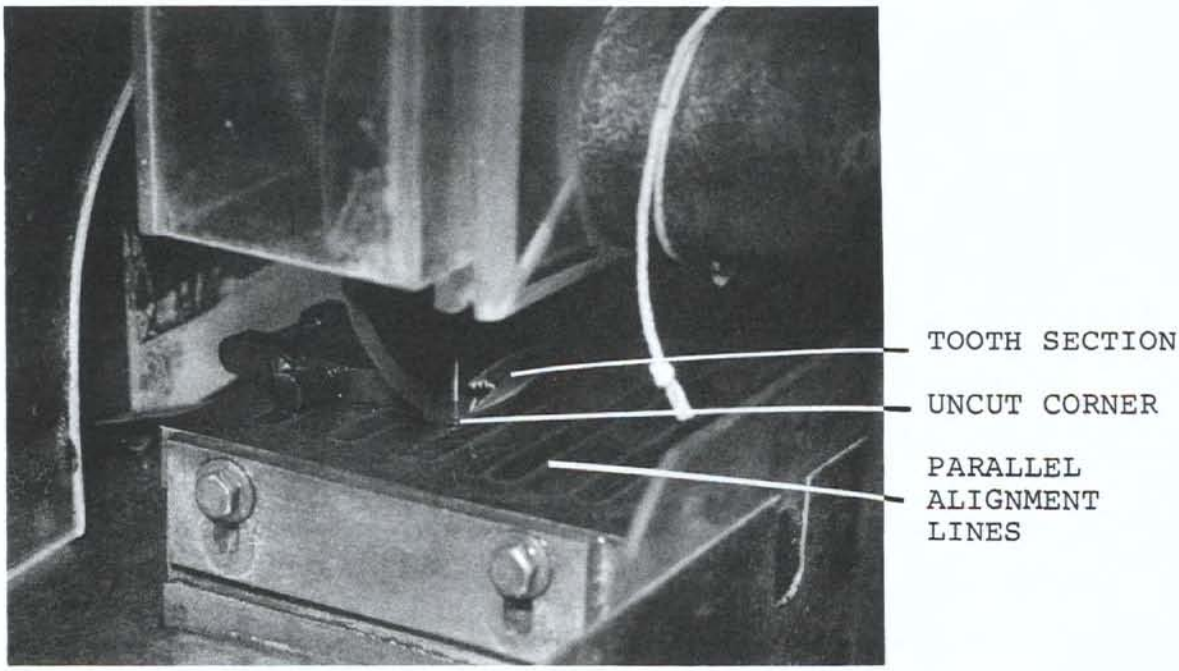
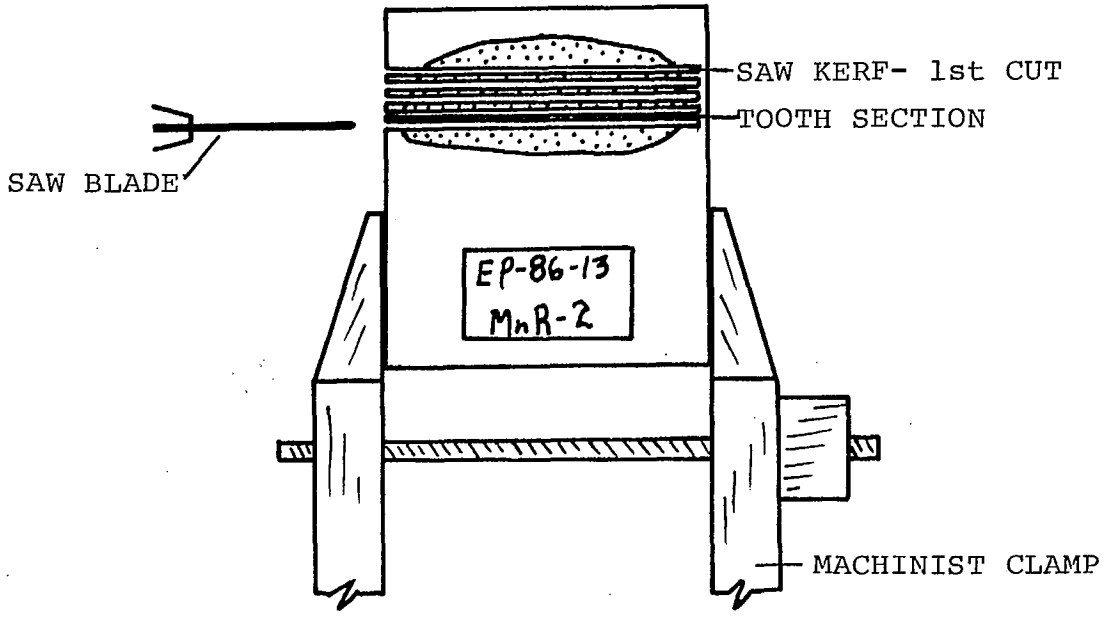
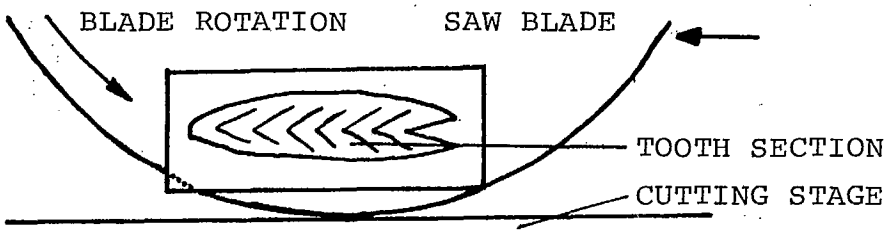


Fig. 8. Tooth block alignment.

TOP VIEW



SIDE VIEW



DO NOT CUT THROUGH BLOCK

LEAVE CORNER TO HOLD SECTIONS TOGETHER

Fig. 9. Cutting sequence.

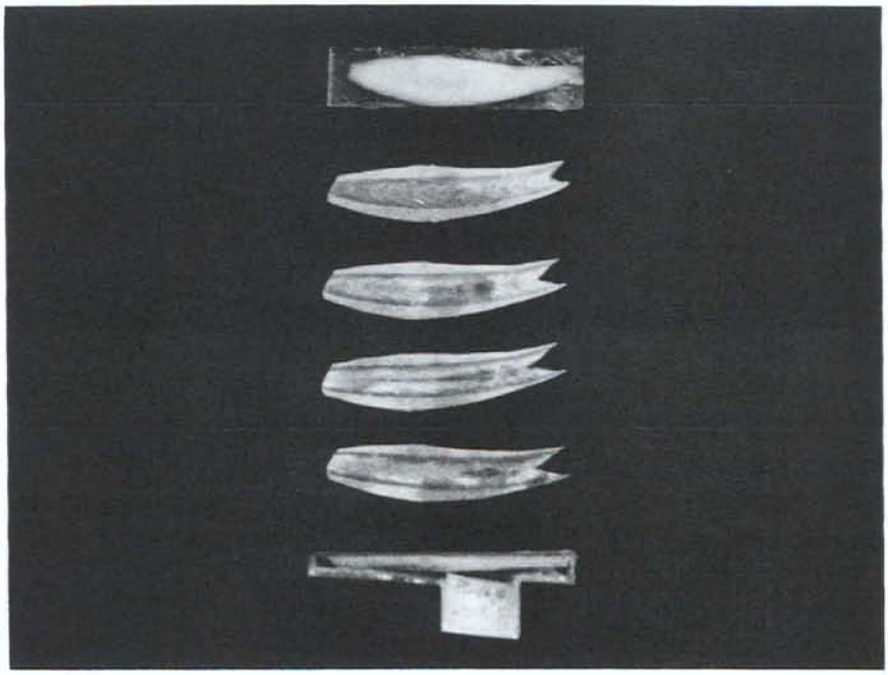


Fig. 10. Sectioned tooth block.

Appendix 1. LIST OF MATERIALS

STEPS INVOLVED IN PREPARING TOOTH THIN SECTIONS

1. Field collection and storage of jaws
2. Extraction of teeth from jaws
3. Mounting teeth for sectioning
4. Sectioning of teeth

LIST OF MATERIALS REQUIRED FOR EACH STEP:

1. Field collection
   - sampling permit
   - whales
   - knife
   - bone/hack saw with coarse blade
   - sample bags
   - labels
   - freezer space
2. Extraction of teeth from jaws
   - fume hood
   - lab coat
   - rubber gloves
   - sturdy knife (15-23 cm blade)
   - soft wood block
   - label tags for jaws
   - light cord
   - boiling pan (will depend on size of jaws and number boiled at one time)
   - hot plate or other heat source for boiling pan
   - large scalpel
   - forceps
   - probe
   - pliers
   - tooth vials (plastic or glass, 20 ml scintillation vials)
   - vial labels
   - 70% solution ethyl alcohol
3. Mounting teeth in resin
   - fume hood
   - lab coat
   - rubber gloves
   - silicone molds
   - clear polyester casting resin
   - catalyst
   - plastic measuring and mixing containers
   - stirring rod
   - pencil
   - forceps
   - paper towels
4. Sectioning of teeth
   - jigsaw
   - thin sectioning machine
   - machinist clamp (Eclipse #410 or 411)
   - 1-2 mm thick metal spacer
   - lab coat
   - safety glasses
   - particle mask air filter
   - forceps
   - elastic bands
   - tooth vials
   - tooth block storage bottles (minimum size 500 ml widemouth bottle)
   - bottle labels
   - 70% solution ethyl alcohol

Appendix 2. SUMMARIZED PROCEDURE

1. FIELD COLLECTIONS
   1. Make up sample bags
   2. Make up sample labels
   3. Expose jaws
   4. Saw off jaws (Figure 1 & 2)
   5. Trim excess tissue and rinse
   6. Bag jaws with labels (Figure 3)
   7. Freeze jaws

2. EXTRACTION OF TEETH FROM JAWS
   1. Thaw jaws in fume hood
   2. Make up labels and attach to cords
   3. Separate mandible halves, label, and refreeze jaw half not required (Figure 3)
   4. Label jaws for boiling (Figure 3)
   5. Remove excess tissue
   6. Cut gum to bone between teeth and down tooth row
   7. Put jaw into boiling water keeping tag out of water
   8. Check after 10 minutes and every few minutes thereafter until tissue comes free, clean off jaw, avoid extensive boiling, maximum 20 minutes
   9. Remove from heat and cool, do not allow to dry out
   10. Label tooth vials
   11. Remove teeth from jaw, clean, and put in vials (Figure 4)
   12. Add 70% ethyl alcohol to vial to cover tooth
   13. Close and store vial
   14. Refreeze or dispose of remaining bone and tissue

3. MOUNTING TEETH FOR SECTIONING
   1. Gather and arrange molds in fume hood (molds made earlier)
   2. Select teeth to be mounted and arrange behind molds ensuring adequate mold space for teeth (Figure 4)
   3. Make up tooth labels on plastic paper in pencil on both sides, put with tooth vial (Figure 4)
   4. Mix up small volume of resin and catalyst
   5. Pour thin layer of resin in each mold (5 mm)
   6. Remove tooth from vial, dry with paper towel (do one tooth at a time)
   7. Arrange tooth and label on resin in mold with the cutting axis straight up and down (Figure 4)
   8. Recheck tooth label with vial label
   9. Repeat 6 to 8 until all teeth are in molds, recheck orientation of axis and label placement
   10. Let stand until jelled (45 minutes)
   11. Add resin to mold until all teeth covered
   12. Let set overnight
   13. Remove block from mold and let harden for a minimum of 1 week in a fume hood or well ventilated area

4. SECTIONING OF TEETH

1. Saw mold block into individual tooth blocks with jig saw (Figure 5a & b)
2. Trim tooth blocks so that each can fit in the machinist clamp (Figure 5b)
3. Assemble sectioning machine (Figure 6)
4. Put splash guards on sectioning machine
5. Turn on and adjust lubricating/cooling water
6. Arrange a tooth block to be cut along tooth axis in the clamp (Figure 7a & b)
7. Align tooth axis and sawblade on sectioning stage (Figure 7c), hold in place by engaging magnet
8. Select section location, then back blade up 2 sections before cutting (if possible)
9. Cut sections at slow stage speed being careful not to cut through to end of block (Figures 8 and 9). Stop stage before changing direction
10. Section until you have the sections you need then cut one more (if possible)
11. Back up blade clear of block and turn off machine
12. Grasp sectioned block to hold sections together and remove clamp and block from stage
13. Check splash tray for any sections that broke free, retrieve and store in section sequence
14. Remove clamp and hold sections and block together with elastic band
15. Break tooth sections free from mounting resin (Figure 10)
16. File sections in original tooth vial, top up alcohol, cap
17. Join remaining block parts with elastic
18. File sectioned tooth blocks in larger bottles in 70% alcohol, label bottles

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