A Guide to Sectioning Otoliths for Age Determination

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ABSTRACT

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This manual describes the methodology, techniques, terminology, materials and equipment used by the Department of Fisheries and Oceans Sclerochronology Lab Program at the Pacific Biological Station in Nanaimo, B.C. to prepare fish otoliths for age determination. Technical preparation errors affect the clarity of annual growth patterns on otolith sections. Poor quality sections impact the quality of age data produced which in turn affects stock assessments and ensuing management decisions for important commercial fisheries. Thus, preparing otoliths for sectioning is labour intensive and requires strict attention to detail. The manual sets out procedures which have evolved and were developed to be the current operational standards for the DFO Pacific Region. A further purpose is to provide information to other agencies and individuals wishing to employ established otolith preparation methods for fish age determination.

RÉSUMÉ

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Le présent manuel décrit les méthodes, les techniques, la terminologie, le matériel et l'équipement utilisés dans le cadre du programme de Pêches et Océans Canada « Laboratoire sclérochronologique » de la Station biologique du Pacifique de Nanaimo, en Colombie-Britannique, afin de préparer les otolithes de poissons en vue de la détermination de l'âge. Les erreurs de préparation technique nuisent à la clarté des schémas de croissance annuelle relatifs aux sections d'otolithes. Des sections de piètre qualité menacent la qualité des données obtenues sur l'âge, ce qui a des effets sur les évaluations des stocks et sur les décisions de gestion concernant les pêches commerciales importantes qui sont prises par la suite. C'est pourquoi la préparation des sections d'otolithes est une opération très longue et requiert une grande minutie. Le présent manuel décrit les procédures modifiées et élaborées pour refléter les normes opérationnelles actuelles en vigueur dans la région du Pacifique de Pêches et Océans Canada. Il vise également à fournir de l'information à d'autres organismes et personnes qui souhaitent utiliser des méthodes éprouvées de préparation des otolithes en vue de la détermination de l'âge des poissons.

1.0. INTRODUCTION

The Sclerochronology Laboratory (SCL) program at the Pacific Biological Station in Nanaimo, B.C. receives numerous annual requests to determine the age of a wide range of commercially important marine fish species from the Pacific Region. On average, the SCL ages around 115,000 – 120,000 fin and shellfish each year. The age data produced is employed by scientists and biologists for stock assessment, management and research purposes. The primary structures used by the SCL for age determination of fish are scales, fins and otoliths (Chilton and Beamish 1982). Scales are generally used to determine the age of short-lived fish such as salmon (1-7yrs) (Hudson and Crosby 2010, MacLellan 2004) and herring (1-12yrs). Fins are used to age moderately long-lived species such as Lingcod (1-21yrs) (Beamish 1981, Cass and Beamish 1983) when otoliths or scales lack clear and/or consistent annual growth patterns. Otoliths are used for long-lived species (>20 years), such as rockfish.

Otoliths are prepared using a variety of methods: no preparation (the surface pattern only is viewed), by burning, by thin sectioning, and by transverse sectioning. The choice of method is determined by species and objectives of the ageing project. The SCL ages about 18,000 fish per year using otolith methods. Currently about 90% are prepared using the burnt otolith technique and the rest are aged without preparation by viewing the surface pattern. Otolith transverse sectioning methods are used for research projects only, i.e. not for production ageing.

As fish approach maturity growth rate reduces significantly as more energy is shifted towards reproduction. All fish bones are considered skeletal, except for otoliths. Skeletal bone growth slows with little to no annual growth deposited on structures such as scales (Power 1978) and fins post maturity. Otoliths, on the other hand, grow as a result of an acellular process (Campana 1999). This means that otoliths continue to grow, forming annual zones, for the entire life of the fish, up to ages greater than 100 years. Understanding otolith formation has been particularly helpful in establishing accurate ageing methods for moderate to long-lived species such as rockfish (*Sebastes*) (Beamish 1979a) and sablefish (*Anoplopoma fimbria*) (Beamish and Chilton 1982). Scale and fin methods significantly under-age such species. The SCL use sagittal otoliths for most moderate to long-lived fish species, especially groundfish. This includes many rockfish and flatfish species as well as sablefish (*Anoplopoma fimbria*) and Pacific hake (*Merluccius productus*).

Most otoliths grow allometrically (Beamish 1979b). They grow in all dimensions until maturity is attained. At that point the otoliths grow thicker on the sulcus or proximal side only. Initially, during the juvenile stage, fish age can be assessed by viewing the distal surface of an otolith. Identifying annuli can be difficult at maturity as annual zones become smaller and more crowded over the otolith, but in particular on the margins of the distal view (Fig 1a). The annuli eventually

become indistinguishable, impossible to count and stop forming on the distal surface completely. Older annual zones are best viewed in cross-section of the otolith, with higher magnification (80 to 100X)(Fig 1b).

In most cases, distinguishable annual zones form for a fish's entire life on the proximal side of the otolith. Before this growth mode was discovered many long-lived fish were under-aged because only the whole surface method was used to produce age by viewing the distal surface. This subsequently affected stock assessments and management decisions (Beamish and Chilton 1982, Leaman and Beamish 1984) as mortality rates were under-estimated. Beamish and McFarlane (1983) reported on the importance of assessing not just precision, but also the accuracy of ageing methods to ensure that best quality age data is used for stock assessments and fisheries research. Since the late 1970's, the technique routinely used by the SCL to prepare otoliths for ageing has been the burnt otolith section method (Chilton & Beamish 1982, MacLellan 1997). This method requires very little preparation and no machinery. However, for a few years before the burnt method was adopted the SCL used the thin section method to production age older rockfish and all Pacific hake otoliths (Beamish 1979b, Chilton & Beamish 1982).

Although not routinely used in the SCL now, thin sectioning of otoliths remains a technique that is used to prepare otoliths with difficult physical characteristics or for special studies. Some otoliths are very small or fragile while others are overly large and thick or oddly shaped making them difficult to break the usual way, with fingers or clippers, for production ageing. In addition, some studies, such as age and growth or chronology based research require measurements of growth zones. These need to be taken along standard planes and growth axes to be relative. Precise sectioning can only be achieved by the use of saws.

This manual describes the methodologies, techniques, terminology, materials and equipment used by the SCL to produce transverse otolith cross-sections. Methods vary depending on the specific application or study requirements. Producing thin sections of otoliths is labour intensive as compared to the scale and otolith burnt section methods. It takes approximately four to five days to prepare and section 100 otoliths, depending on the preparation method. The methods that do not involve mounting the otoliths in an epoxy are much quicker. Some of the techniques are similar to how the SCL prepares and sections fins for ageing (Little et al. 2012). The primary purpose of this manual is to promote consistent otolith preparation and sectioning methodology and to provide instructions for training technicians in the SCL. Specific materials, equipment and product names, referred to in this manual, are not an endorsement by Fisheries and Oceans Canada. Similar equipment and materials may be substituted.

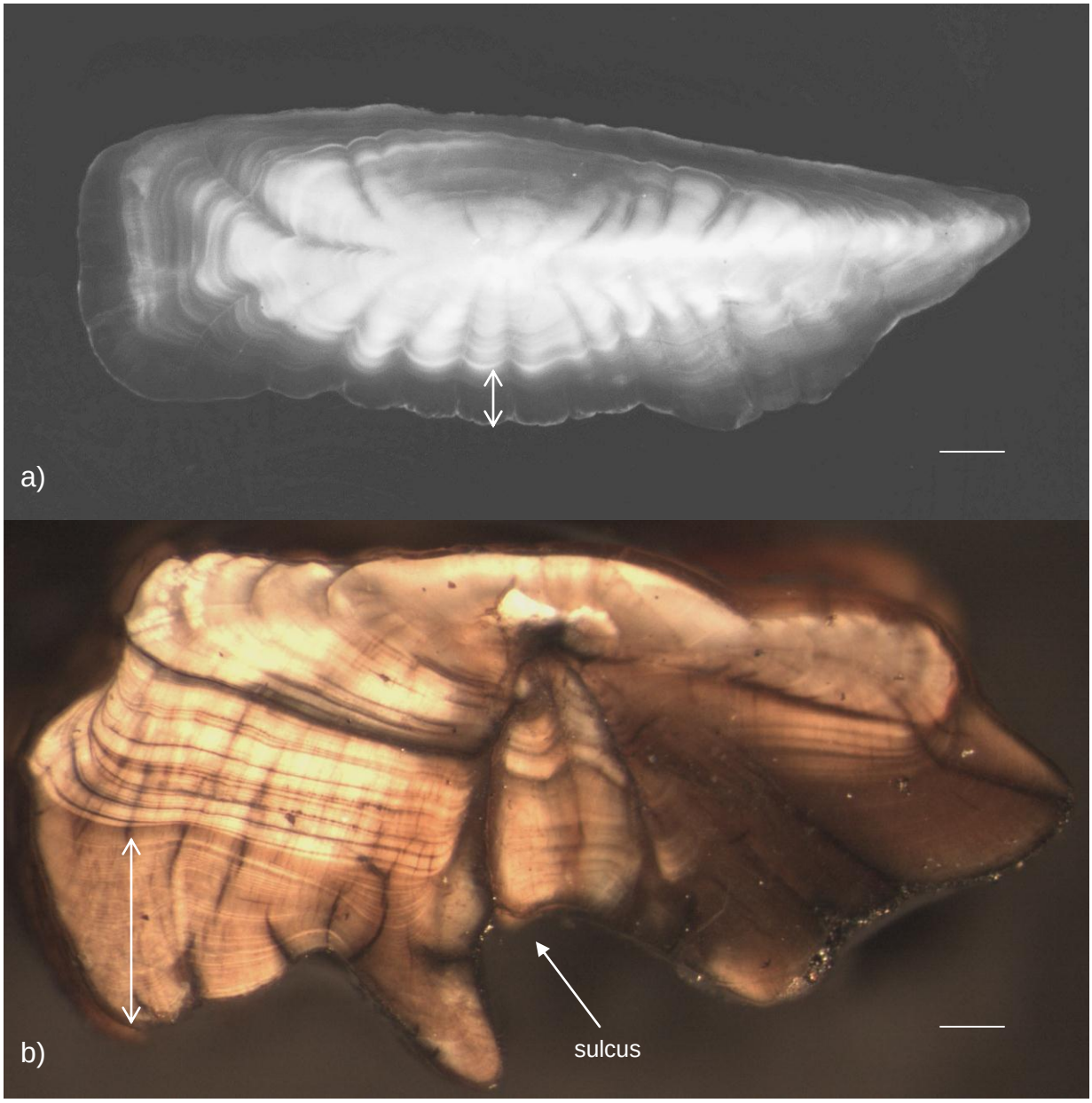

Figure 1**.** Different views and preparations of the same sablefish (*Anoplopoma fimbria*) otolith. The double-headed arrows point out equivalent older growth on the otolith margins. a) Distal whole surface view (scale bar = 0.46cm). Even under a microscope the older annuli cannot be distinguished on the dark edges of surface margins. b) Burnt transverse cross-section view (scale bar = 0.15cm). Under high magnification (100X), growth on the proximal/sulcus side of the burnt section reveals individual annuli for more accurate counts.

2.0. METHODS

The SCL receives and stores otoliths in a plastic tray called Tray bien® (Fig. 2). A tray is divided into100 cells, each of which can accommodate a pair of otoliths. Sample information is written on each tray. To discourage fungal growth during storage, a small amount of anti-fungal solution is added to each cell containing otoliths. The solution is prepared by dissolving one teaspoon of thymol crystals in a small amount of alcohol and then added to one gallon of mixed glycerin and water (50:50). This solution is diluted enough that it has been assessed not to be hazardous under the Workplace Hazardous Material Information System (WHMIS).

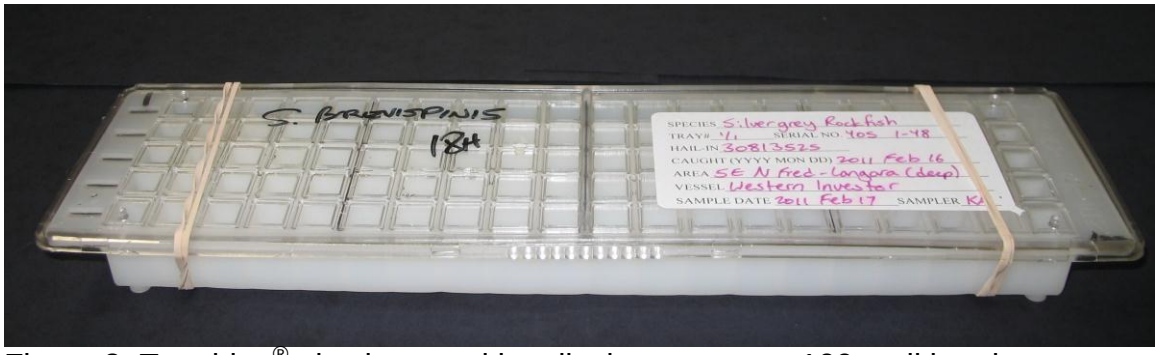

Figure 2. Tray bien® plastic tray with cells that can store 100 otolith pairs.

Technical (preparation) errors that affect age estimates can be introduced if otoliths are not properly prepared, especially with regards to orientation, for sectioning. A good eye is needed to properly position otoliths in epoxies and saw chucks to ensure that the otolith is cut perpendicular to its long axis to produce best quality sections with clear alternating translucent and opaque growth zones (Fig. 3a). Oblique cuts (Fig. 3b) can distort the shape and size of annual growth zones and may blur patterns on sections, which can lead to mis-measurement or mis-ageing. Section thickness can also affect pattern clarity. In general, the SCL produces cross-sections for smaller and younger otoliths thinner than for bigger older otoliths. When producing multiple sections per otolith, the SCL varies thickness slightly, by eye, rather than using specific measurements. The SCL has not established specific section thicknesses for each species. If multiple sections per otolith are produced measurements vary from about 0.45 – 1.35mm in thickness, with 0.87mm being the average. Variation ensures a range for viewing patterns that can be useful. The recommendation is that each agency should establish their own optimum thicknesses for the otolith sections of each species they work with.

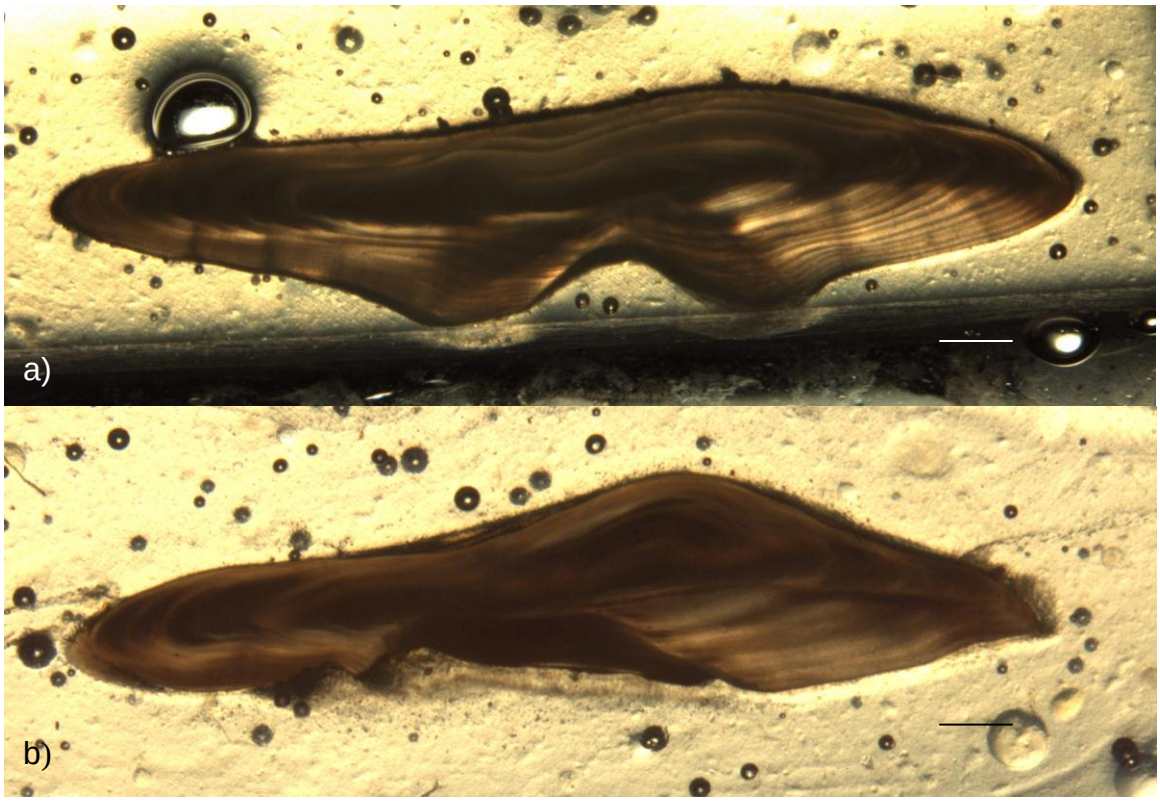

Figure 3. Examples of hake (*Merluccius productus*) otolith thin sections comparing quality of the preparation. a) Perpendicular cut resulting in distinct alternating translucent and opaque zones for ease of counting. b) Oblique cut resulting in a blurred annual growth pattern. Scale bar = 0.40cm.

In most cases, otoliths are sectioned with either a coat of epoxy or embedded in an epoxy plug. This provides support to the otolith preventing fragile parts from breaking off as well as a better grip in the sectioning machine chuck to avoid movement during cutting. Epoxies used for sectioning otoliths need to be translucent once cured so that the operator can see where to cut the otolith. The SCL uses two types of epoxy. Hysol® (parts A&B) is a high viscosity epoxy and Epo-thin® is a low viscosity epoxy used with molds. Hysol® is used to support large and small otoliths that can be sectioned using either the Bronwill® high-speed sectioning machine or the Buehler® Isomet™ low-speed saw. Epo-thin® is used for smaller otoliths that are set up in an epoxy plug for sectioning by the low-speed saw. Some otoliths are often too small to be sectioned by the high-speed saw which is more powerful and has a fairly thick cutting blade.

2.1. OTOLITH PREPARATION

2.1.1 Method 1: Using epoxy molds to encase otoliths

The SCL uses epoxy molds and a low viscosity epoxy to prepare small otoliths less than 10-15mm in length. The molds are made of flexible silicone rubber facilitating removal of the otoliths once the epoxy plug has cured or hardened. Only the chuck of the low-speed saw will accommodate otoliths prepared this way for sectioning. All mixing and application of epoxies must be done inside an approved exhaust system (fume hood) to remove toxic fumes and personal safety equipment must be worn to avoid contact with skin and clothing.

2.1.1.1 Equipment & Materials:

- otolith storage container (e.g. Tray bien®)
- water
- task wipes (e.g. Kimwipes®)
- paper towels
- soft pencil
- molds (e.g. Ted Pella - silicone rubber- double ended)
- epoxy resin & hardener (e.g. Epo-thin®)
- forceps
- probe
- disposable container
- Popsicle sticks
- 2 syringes with measuring marks
- 1 eye dropper
- paper towel
- fine tipped permanent marker pen
- coin envelopes (un-gummed)
- health & safety: fume hood with current inspection label
- personal protection equipment: lab coat, disposable latex or nitrile gloves

2.1.1.2 Procedure

1. Select one otolith from each pair. For consistency choose the left or right.
2. Thoroughly wash each otolith in clean water to remove all glycerin storage mixture and tissues. Wipe dry with Kimwipes® and place into a separate labelled, clean and dry Tray bien® cell.
3. View the cleaned otolith under a dissecting microscope and mark the cutting line through the nucleus on the distal side with a soft lead pencil to provide a guide for sectioning (Fig. 4).
4. Return the marked otoliths to the dry tray ready for the epoxy procedure.

5. Design a logical sequential numbering system for the mold and put the epoxy into the cells starting at the first position. The SCL uses silicone molds with 21 cells that are pre-numbered (Fig. 5).
6. Turn on the fume hood to high exhaust and adjust window height to the recommended level (marked on the side of the fume hood). Carry out all epoxy procedures inside the hood to avoid breathing toxic fumes.
7. Measure a small amount of Epo-thin® resin & hardener (e.g. ~4ml will fill all cells in one 21 cell mold) into a disposable container by using syringes, one each for resin and hardener. Measure out five parts resin to two parts hardener. Mix well using a Popsicle stick.
8. Use an eyedropper to place about five drops of Epo-thin® into each cell to partially fill them (about half full). This will provide a platform to place the otoliths. Let epoxy harden for about three hours until it is semi-hard or tacky. Clean the eyedropper with warm soapy water. Epo-thin is a liquid and doesn't thicken quickly.
9. Remove the otoliths from the Tray-bien®, in the correct order. Place each, sulcus (proximal) side down, into the epoxy in the mold with the pencil cutting line facing up. This is a very important step. Take the time to properly orient the otolith so that it lies 'flat', i.e. is not tipped longitudinally, and that its long axis is parallel to the long axis of the mold. This will help ensure a perpendicular, rather than an oblique, cut of the otolith and will produce a good quality section.
10. Once all otoliths have been positioned in the half-filled mold, carefully cover each of the otoliths with about 5 drops of epoxy to top up the mold. Watch that the addition of epoxy does not affect the proper orientation of the otolith in the mold. Readjust with a probe if necessary.
11. Let cure overnight. The epoxy is cured hard enough when a thumbnail cannot make a dent.
12. Use a permanent marker to label coin envelopes. Record all sample information on the first envelope such as: species name, species abbreviation and fish number, first and last fish number in sample, vessel name, area and date caught. Write the species abbreviation (e.g. Sablefish is SB) followed by the fish number, e.g. SB1, SB2…, at the top of each envelope for ease of identification (Fig. 6).
13. Remove the epoxied otoliths by twisting the silicone mold. Place each otolith into its respective numbered envelope to store and organize for sectioning. Make sure that the fish ID number for the epoxied otolith matches up with the number on the envelope.

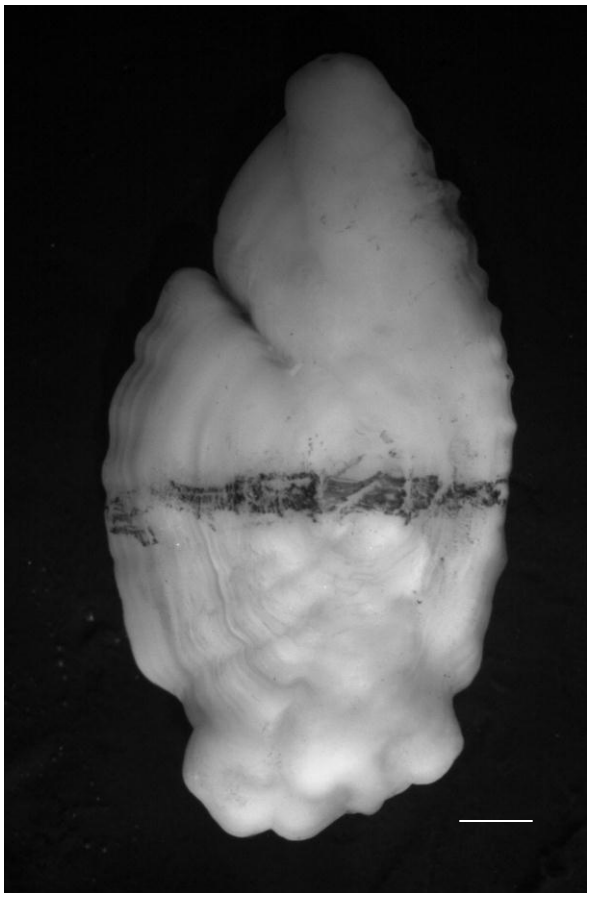

Figure 4. A Pacific Ocean Perch rockfish (*Sebastes alutus*) otolith distal surface marked through the nucleus with a transverse reference cutting line by a soft lead pencil. Scale bar = 0.12cm.

Figure 5. Silicone mold used to mount small otoliths in epoxy. Sablefish otoliths are shown in the 2 bottom-left cells. Scale bar = 1.06cm.

Figure 6. All sample information is recorded on the first storage envelope and only the fish ID number is written at the top of subsequent envelopes.

2.1.2 Method 2: Making beds of epoxy to encase otoliths

This method can be used for both large (>10mm) and small otoliths. The SCL uses Hysol® epoxy for this method as it requires a high viscosity product. Preparation involves forming a bed of epoxy to the desired size and shape to embed the otolith. Either the high-speed Bronwill® or low-speed Buehler® Isomet saw can be used for sectioning. All mixing and application of epoxies must be done inside an approved exhaust system to remove toxic fumes and personal safety equipment must be worn to avoid contact with skin and clothing.

2.1.2.1 Equipment & Materials

- large tray
- Parafilm®
- epoxy resin & hardener (e.g. Hysol® Epoxy A&B)
- forceps
- probe
- soft pencil
- disposable container
- Popsicle sticks
- spoon or spatula
- disposable measuring cup
- task wipes (e.g. Kimwipes®)
- paper towel
- permanent marker or waterproof pen
- coin envelopes (un-gummed)
- health & safety: fume hood
- personal protection equipment: lab coat, disposable latex or nitrile gloves

2.1.2.2 Procedure

1. Cover a board with waxy Parafilm® to prevent the epoxy from sticking to the board once cured.
2. Draw a grid system on the Parafilm® (Fig. 7) and label the squares using a permanent marker. Input sample information in the top left hand corner square. Number the rest from left to right in rows and top to bottom. Make sure the otolith ID numbers from the Tray bien® co-ordinate with the numbers on the board.
3. Turn the fume hood on to high exhaust and adjust window height to the recommended level (marked on the side of the fume hood). Do all epoxy procedures inside the hood to avoid breathing toxic fumes.
4. In the fume hood, use separate spoons/spatulas to measure resin and hardener into a disposable container. Separate utensils are needed to avoid cross-contaminating resin and hardener containers. Stir well with Popsicle stick for 2-3 minutes. The mixture should be worked to an even opaque appearance. To avoid waste, try to estimate the amount of epoxy needed to make beds for one board of otoliths. Start with a small amount of epoxy and make more if needed.
5. Clean each utensil with a separate piece of paper towel. Leave used paper towel in the fume hood and place in the garbage at the end of the day.
6. Work with one row on the board at a time. Spread a bed of epoxy (Fig. 8) on each square on the grid. It should be roughly 6cm in length to fit into the saw chuck and accommodate efficient and safe cutting action for the saw blade. The encased otolith must project about 2cm or more out from the chuck to ensure that the blade and its flanges do not make contact with the metal chuck.
7. Remove the otoliths from the Tray-bien®, in the correct order. Dry the otolith by wiping off the glycerin storage medium with a task wipe.
8. If needed, use a soft pencil to mark the cutting plane through the nucleus of the otolith to guide sectioning. Either otolith may be used for production ageing, but when measurements are required use the same otolith (normally the left) for the whole project.
9. Place the otolith into the bed of epoxy sulcus side down. This is a very important step. Take the time to properly orient the otolith so that it lies “flat”, i.e. not tipped longitudinally, and that its long axis is parallel to the long axis of the epoxy bed. This will help ensure a perpendicular, rather than oblique, cut of the otolith and will produce a good quality section.
10. Use a Popsicle stick to cover each otolith with epoxy. Ensure that no surface is missed (Fig. 9). Make certain that the addition of epoxy does not affect the proper orientation of the otolith in the epoxy bed. Readjust with a probe if necessary.
11. Let the epoxy cure overnight or until a thumbnail does not make a dent.

12. Use a permanent marker to label coin envelopes. Record all sample information on the first envelope such as: species name, species abbreviation and fish number, first and last fish number in sample, vessel name, area and date caught. Write the species abbreviation (e.g. Sablefish is SB) followed by the fish number, e.g. SB1, SB2…, at the top of each envelope for ease of identification (Fig. 6).
13. Once cured, remove the epoxied otoliths from the board. Place each otolith into its respective numbered envelope to store and organize for sectioning. Make sure that the fish ID number for the epoxied otolith matches up with the number on the envelope.

Figure 7. A wooden board covered with Parafilm®. Large otoliths have been embedded in epoxy and then placed in consecutive order onto the numbered grid. Sample information is recorded at top left and each grid is labelled with the otolith ID number.

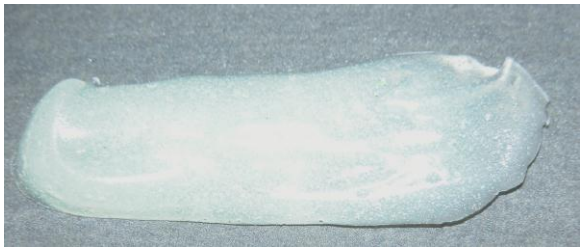

Figure 8. Uncured bed of epoxy shaped to place an otolith for encasement.

Figure 9. Close-up of a rockfish otolith embedded and completely covered in epoxy. Scale bar = 1.00cm.

2.1.3 Method 3: Mounting onto tags with thermal plastic cement/glue

The thermal plastic glue method works for both small and large otoliths that are to be sectioned by the low-speed saw and the otoliths do not need to be completely embedded. Instead, the otoliths are attached to a cardboard tag with the glue which is then inserted into the Buehler® Isomet™ saw chuck for sectioning.

2.1.3.1 Equipment & Materials

- hotplate
- thermal plastic cement or glue
- cardboard 2.3cm x 4.2cm tags
- ruler
- pencil
- forceps
- probe
- small hammer
- metal or plastic spatula
- paper towel
- water

2.1.3.2 Procedure

1. Cut thin, but firm, cardboard, e.g. 0.3mm thick, into rectangles measuring 2.3cm x 4.2cm to make a tag to glue the otolith onto. The tag must fit snuggly into the guide grooves of the Buehler® Isomet™ slow-speed saw chuck.
2. Slide the blank tag into the chuck to mark cross-hairs on it. Use the Allen wrench to adjust the chuck to about a 45° angle. Gently set the chuck down onto the blade to touch the centre of the cardboard tag. Mark the horizontal centre axis of the cardboard with a pencil where the blade hits. Use the micrometer dial on the saw arm to centre the tag over the saw blade. The angle of the chuck may also need a slight adjustment. Remove the tag and use a ruler to draw two intersecting perpendicular lines, one across the tag through the pencil mark and a vertical line down through the center (Fig. 10). These are used to line up the otolith for sectioning. Use this tag to mark the cross-hairs of the rest of the tags.
3. Assemble the equipment (hotplate, hammer) and materials (thermal plastic cement) to glue the otolith to the tags (Fig. 11).
4. Use a soft pencil to mark a section line through the nucleus of the otolith.
5. Heat a hotplate to 204°C or 400°F.
6. Break off some small chips of the cement using a small hammer.
7. Put a few of the chips of cement onto the center of the cardboard tag and place on the hotplate to melt.
8. Use forceps to place the otolith onto the melted cement sulcus side down with the nucleus on the cross-hair (Fig. 12). This is a very important step.

Take the time to properly orient the otolith so that it lies “flat”, i.e. is not tipped longitudinally, and that its long axis is parallel to the short axis of the tag. This will help ensure a perpendicular, rather than oblique, cut of the otolith and will produce a good quality section.

9. Remove the tag with glued otolith from the hotplate to cool with forceps. The cement hardens quickly once removed from heat. If the otolith is not oriented correctly replace the tag back onto the hotplate to re-melt the cement. Adjust the otolith’s position with a probe adding more cement chips if needed.

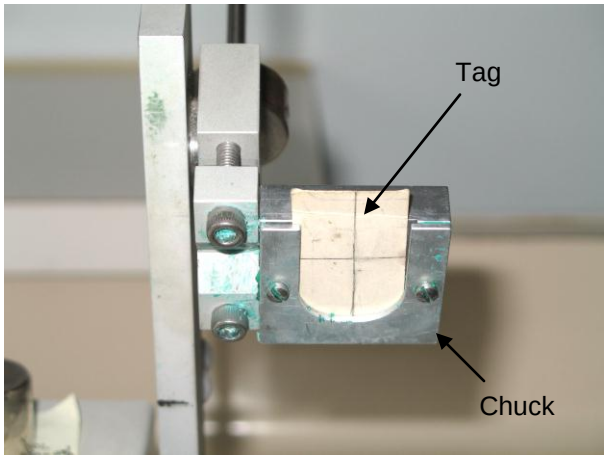

Figure 10. Close-up of low-speed saw support arm and chuck with cardboard tag marked for lining up an otolith for sectioning.

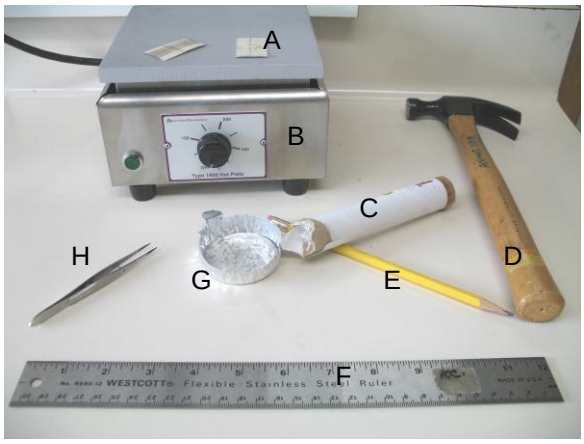

Figure 11. Equipment and materials used to fasten otoliths onto cardboard tags with thermo plastic glue/cement: A-card board tags, B-hotplate, C-thermal plastic cement, D- hammer, E-soft lead pencil, F-ruler, G-dish for crystal chips broken from thermal plastic cement stick, H-forceps.

Figure 12. Marked sablefish otolith attached with thermal glue to a cardboard tag. The otolith nucleus is lined up with the drawn cross-hairs.

2.1.4 Method 4: No otolith preparation

This method is used during production ageing when otoliths, due to their size (thick, small) or shape (round), cannot be properly broken through the nucleus manually with fingers or clippers. Otoliths are not encased in epoxy or glue. Instead they are pushed into plasticine on the saw chuck of the low-speed saw to be cut in half or bisected. As with other methods it is important to ensure that the otolith is aligned properly to ensure perpendicular cuts. The method may also be used for some special studies where a standard cutting axis is required (e.g. growth measurements) and the otoliths are robust enough that they do not need to be epoxied.

2.2 OTOLITH SECTIONING

Sectioning requirements and application dictate the selection of machinery employed to produce otolith sections. The SCL has two types of sectioning saws: the low-speed Buehler® Isomet™ saw and the high-speed Bronwill® TSM Universal Model 77 bone sectioning machine. The Buehler® saw can either bi-sect otoliths or cut one or more sections. Thin cross-sections can be produced through a single or double saw blade arrangement. It is equipped with a micro-measurement feature along with several other adjustable parts. This provides flexibility to manipulate and cut sections to accurate thicknesses. The Bronwill® saw is a more powerful and faster automated machine that can be used to section otoliths as well as other boney structures. This saw, has only one blade and does not have the ability to accurately control section thickness (gross estimate only).

The SCL uses various methods to prepare otoliths to be cross-sectioned. Three preparations can be cut using the Buehler® saw and one with the Bronwill®. The Buehler® is used when otoliths have been embedded in epoxy molds, glued to tags with thermal plastic cement or when non-epoxied otoliths are simply stuck onto the plasticine-covered chuck. The Bronwill® saw is only used to section otoliths that have been embedded in an epoxy bed.

2.2.1 Low-speed saw methods

The Buehler® low-speed saw (Fig. 13, 14) is fairly versatile. The operator should become familiar with the working parts of the machine before use. This saw comes with a variety of chucks to hold specimens. The SCL only uses one with the otolith fixed either with plasticine or to a cardboard tag (described in the otolith preparation section).

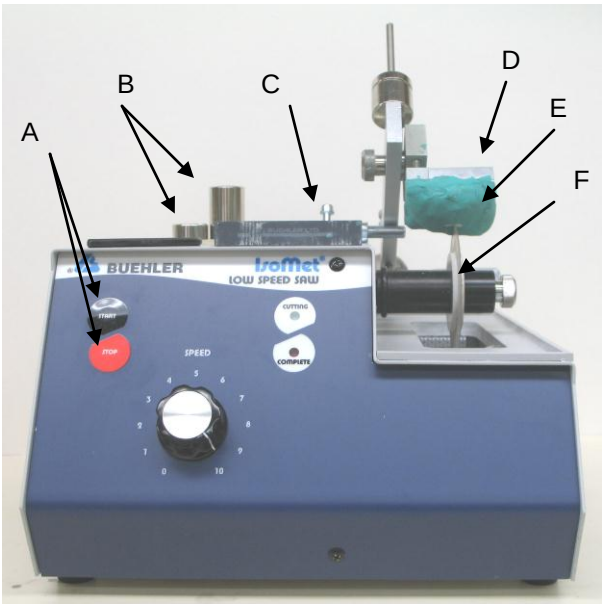

Figure 13. Front view of Buehler® Isomet™ low-speed saw: A-start/stop buttons, B weights, C-slide lock, D-chuck, E-plasticine, F-blade.

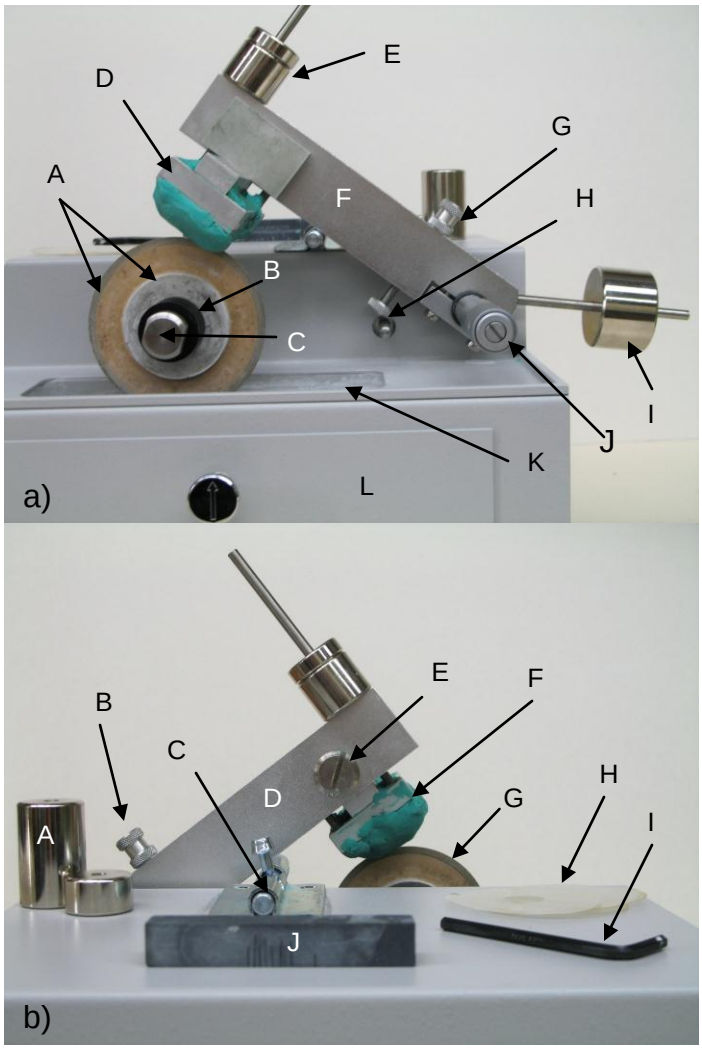

Figure 14. Close up opposite side views of the Buehler® Isomet™ low-speed saw with parts labeled. Note that the chuck is positioned at about 45° to the horizontal for the best cutting angle.
a) Right side view showing: A-blade with flange, B-bushing, C-thumb screw, D-chuck, E-arm weights, F-support arm, G- cut off switch adjustment screw, H-cut off switch, I-adjustable arm counter weight, J-micrometer head, K-water pan opening, L-lubricant pan access door.
b) Left side view showing A- arm weights, B-cut off switch adjustment screw, C-slide lock, D-support arm, E-blade angle screw, F-chuck, G-blade, H-double blade spacers, I-Allen wrench, J-dressing stick.

The saw can be set up with either one or two blades for making cuts. Other than how the otolith is positioned for the first cut, sectioning procedures are the same. Cutting with one blade is slower, necessitating numerous passes of the blade through the otolith and will produce one or more sections with each side of a section being cut individually. It takes two cuts to produce one thin section and the width of each section can be accurately regulated by adjusting the micrometer head control or dial between cuts. When estimating where to make the cuts on the otolith to the desired section thickness the operator must be sure to take the width of the saw blade into account. A single section can be cut more quickly, with one cutting action, by doubling up the blades on the machine. In this case, the width of the section is regulated by a spacer(s) of specified thickness placed between the two blades.

Quality of the otolith cross-section can be affected by otolith orientation, condition of the plasticine (if used), amount of weight placed on the chuck arm, angle of the saw blade approach to the chuck and speed of blade rotation. Oblique cuts (Fig. 3b) with unclear growth zones are most commonly produced by incorrect otolith

positioning and/or the otolith shifting in the saw chuck. The otolith must be oriented so that its longitudinal axis is perpendicular to the saw blade. It takes a practiced eye to do this and the operator must watch to ensure that the otolith does not shift during sectioning. In addition, control through slower cutting speed and less weight usually produces better quality sections. It is recommended to use less, rather than more, weight on the chuck arm as the heavier the load the greater the likelihood there will be damage to the section or blades. Damage manifests as chipped or oblique sections. Too much weight and/or blade rotation speed can cause the otolith to shift in the chuck or the blade to push, rather than cut, through the otolith. The most efficient cutting action is produced when the chuck is oriented at about a 45°angle to the horizontal (Fig. 14). This ensures that the cut begins on both the dorsal and ventral edges of convex otoliths at approximately the same time. If plasticine is used to hold the otolith to the chuck the operator should be prepared to replace it at intervals. If large numbers of otoliths are being sectioned the water lubricant may soften the plasticine causing the otolith to shift during sectioning and may result in oblique cuts.

2.2.1.1 Sectioning mold epoxied otoliths

This method is employed when precise measurements for otolith section thickness and/or growth zone size are required or when otoliths are small or fragile and need to be epoxied for support.

2.2.1.1.1 Equipment & Materials

- low-speed saw (e.g. Buehler® Isomet™)
- diamond studded blades (e.g. Buehler® wafering blades #11-4243)
- plasticine
- water
- dish soap
- forceps
- frosted end glass slides
- fine permanent marker
- slide drying boards & rack
- personal protection equipment: eye protection

2.2.1.1.2 Preparation

1. Label slides using a fine permanent marker (Fig. 15). Record all sample information on the first slide, such as: species abbreviation (e.g. Sablefish = SB) and fish number, species name and first and last fish number in sample, vessel name, area and date caught. The fish ID number must be written at the top of all slides so the number can be seen when the slides are stored in the slide box. All subsequent slides need only be labeled with species and fish number (e.g. SB1, SB2…).

2. The operator should become familiar with working parts of the Buehler® Isomet™ low-speed saw (Figs. 13, 14).
3. Add water to the saw lubricant pan located directly below the blade to a level where the water is touching the blade. The water reduces friction during sectioning. A few drops of dish soap can help to reduce friction further. There is a sample basket located inside the pan, which is used for retrieving (lifting out) any sections or otolith parts that may fall into the water. To access the pan open the side door located on the side of the sectioning machine by turning the knob. To remove the pan, lower the support tray by first lifting it up and then outward. Return the tray to its original position and close the door.
4. Decide if one or two saw blades are preferred to make sections (Fig 16). If the two blade method is desired:
    a. Remove the thumb screw, bushing and outer flange located at the end of the shaft drive for the blade.
    b. Add a plastic or metal spacer of determined thickness to the shaft outside the inside blade (Fig. 17). If one spacer is not thick enough add more. The SCL uses plastic acetate for spacers. To make a spacer trace the outline of a flange and shaft hole onto the material and cut out the round shape.
    c. Add the second blade and replace the flange, bushing and retighten the thumb screw.
5. A slide lock is located on the top of the sectioning machine, placed near the top of the support arm that holds the chuck up. This prevents the support arm and chuck from falling onto and damaging the expensive saw blade when not in use. This is a modification added by the SCL as the blades are very fragile and will chip, crack or warp with very small amounts of force. Only disengage the slide lock to cut sections. Keep engaged at all other times.
6. Mold a large piece of plasticine onto the surface of the chuck (Fig. 16). Ensure it is thick enough to provide a generous cushion between the otolith and the chuck to keep the blade from hitting the metal chuck once the cut has been completed.
7. Ensure that the chuck is firmly attached to the arm to prevent movement during sectioning. Tighten the two screws with an Allen key if needed.
8. Adjust the cut-off switch mechanism located on the top of the support arm. When set it will automatically shut off the saw when a section is cut to a specified depth. This prevents blade damage caused by making contact with the metal chuck.
9. Add weights to the support arm as needed. There is a shaft located at the forward end of the support arm that accommodates weights to provide some downward pressure on the blade during sectioning. Four weight sizes are available starting at 25mg with each larger one heavier by 25mg.
10. Mark the position (plane) of the blade(s) in the plasticine for reference by releasing the arm slide lock and gently lowering onto the blade. Lift back up and re-engage the slide lock.

SB1 Sablefish 1-50 Royal Viking Area 3C 2002 Jan.10	

SB 2	

Figure 15. Label slides with sample information. The 1st slide (top) should record full sample information. Subsequent slides (bottom) need only the fish ID number marked at the top.

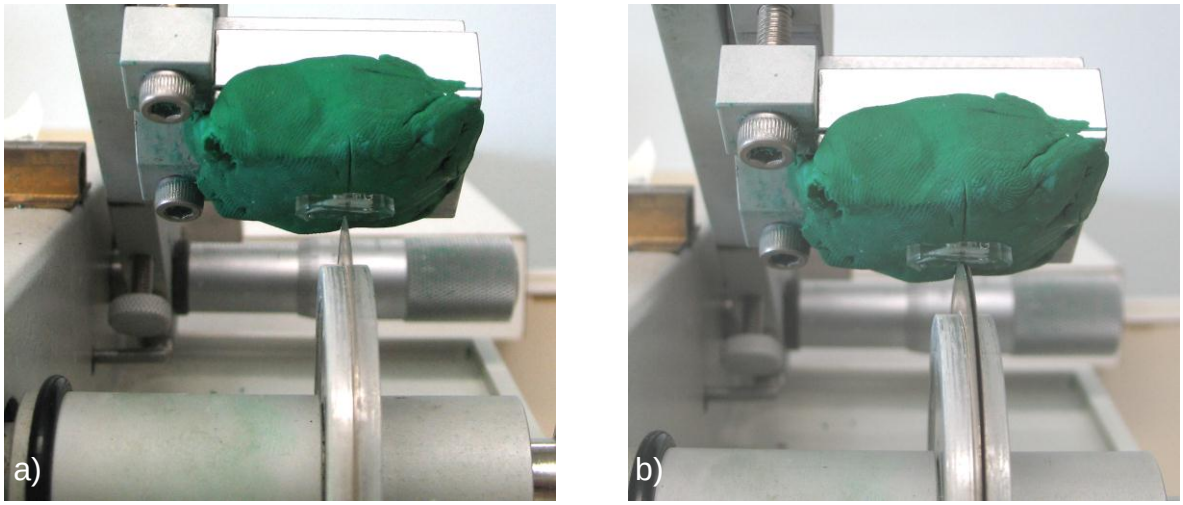

Figure 16. Single or double blades can be used to produce otolith sections on the Isomet® saw. Green plasticine has been molded onto the chuck to hold the otoliths. a) One blade configuration. b) Double blade configuration.

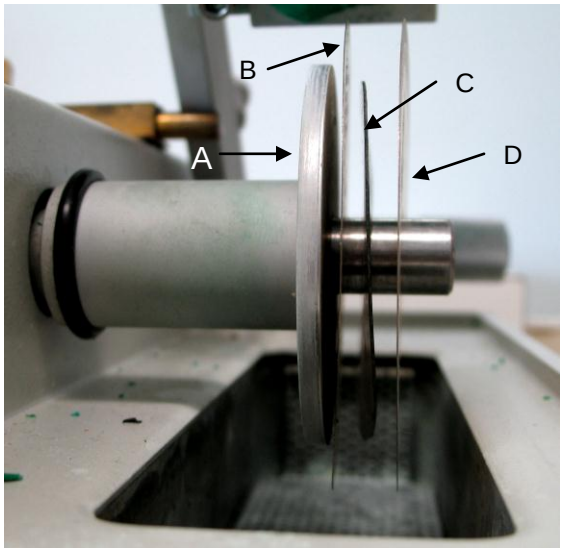

Figure 17. Photo illustrating how the A-inside flange, B-saw blade, C-spacer and D-2nd saw blade are placed on the shaft to make a section with a single cut. Missing from the photo are the second flange, the bushing and the thumb screw.

2.2.1.1.3 Procedure

1. Place the otolith into the plasticine matching the otolith centre mark with the blade mark for a reference cutting axis. Push the otolith just deep enough to be secure against movement during sectioning. It may be necessary to loosen the chuck to rotate it towards the eye to easily affix the otolith.
2. Align the 1st cut by setting the chuck to about a 45° angle to the horizontal for an efficient cutting angle to the blade (Fig. 14). Adjust the horizontal position of the arm by turning the micrometer dial located at the back right corner of the saw, on the support arm.
    a. One blade method: Position the saw blade to the right of the otolith centre mark or nucleus to start. This takes into account the width of the blade for precise sectioning. Multiple cuts and sections require repositioning the blade with the micrometer dial clockwise for subsequent cuts. Sections can be cut as thin as 0.5mm and as thick as 1-2mm or more, as desired.
    b. Double blade method: Line up the blades to straddle the otolith centre mark or nucleus to cut one section. When using two blades only one cut is required with the spacer determining section thickness.
3. Push the start (black) button located on the front of the saw to activate rotation of the saw blade.
4. Set and control the saw blade rotation speed with the dial located on the front of the machine. Start with a lower setting (4 – 6) and speed up (7-8) once the cut is initiated. Disengage the slide lock and start cutting by lowering the otolith and chuck gently onto the blade.
5. Be prepared to slow down speed as the cut nears completion. If the adjustment cut off switch is set the machine will stop automatically. Or, the operator can stop the blade by pressing the (red) stop button.
6. Turn the machine off after the 1st cut, lift chuck off the blade by the support arm and put slide lock on.
    a. One blade method: Rotate the micrometer dial to move the chuck inwards, the desired section thickness, to set up for the next cut. Disengage the slide lock, lower the otolith onto blade, press the start button and begin the 2nd cut. If more than one section is desired repeat process. Sections may have to be retrieved out of the water pan with forceps.
    b. Double blade method: Sectioning is complete. If the section becomes lodged between the two blades remove the thumb screw, bushing, flange and outer blade to retrieve it. The SCL makes and uses an acetate tool, cut out to form a hook at the end, to fish out the section to avoid disassembly of blades.
7. Place the section(s) onto the corresponding numbered slide, last cut side facing up. Multiple sections should be oriented all in the same direction – same side up in the order cut, left to right on the slide (Fig. 18). Store

remaining otolith parts back into the correctly numbered envelope in case re-sectioning is required.

8. Repeat steps for each otolith and section them in sequential order.
9. Place slides with sections onto slide drying boards and place into drying rack when each board is full (Fig.19).
10. Let sections dry overnight before adhering to slides.

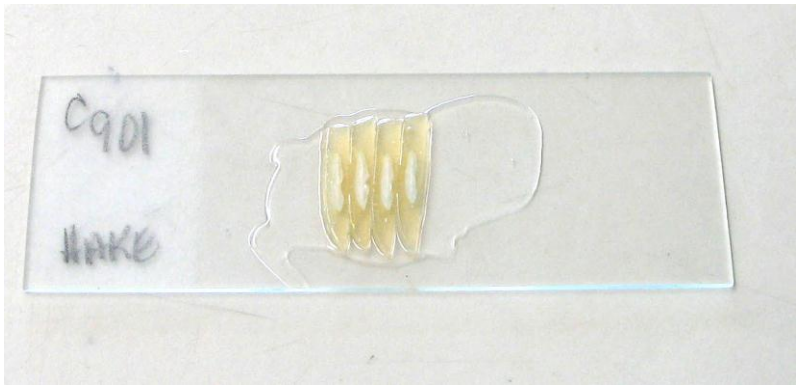

Figure 18. Proper orientation of otolith sections on glass slide.

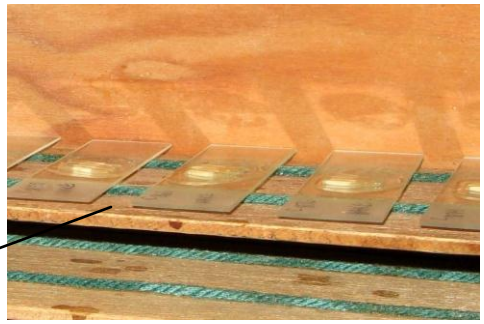

Figure 19. Drying rack with otolith sections on glass slides placed in numerical order on drying boards. The close up at right shows slides in more detail.

2.2.1.2 Sectioning otoliths mounted with thermal plastic cement/glue

This method is a very quick otolith mounting method employed when precise measurements (e.g. section thickness and/or growth zone size) are required and the otoliths are robust enough that they need not be encased in epoxy.

2.2.1.2.1 Equipment & Materials

- low speed saw (e.g. Buehler® Isomet™)
- water
- forceps
- frosted end glass slides
- fine permanent marker
- slide drying boards & rack
- personal protection equipment: eye protection

2.2.1.2.2 Preparation

Follow preparations as for sectioning mold epoxied otoliths with the low-speed saw. The same chuck is used but no plasticine is applied. Instead, otoliths are mounted onto cardboard tags and placed into the chuck. It is particularly important to set the adjustment cut off switch correctly to avoid damaging the saw blade on the metal chuck if it cuts through the tag.

2.2.1.2.3 Procedure

Follow steps as for mold epoxied otoliths, except for those involving plasticine, and add these steps:

1. Use the cross-hairs of the cardboard tag to line up the saw for cutting based on use of a single or double blade (Fig. 20).
2. Remove the tag from the chuck when the section is cut.
3. If the section remains attached to the tag, carefully bend along the cut lines and gently remove it with forceps and place on labelled glass slide.

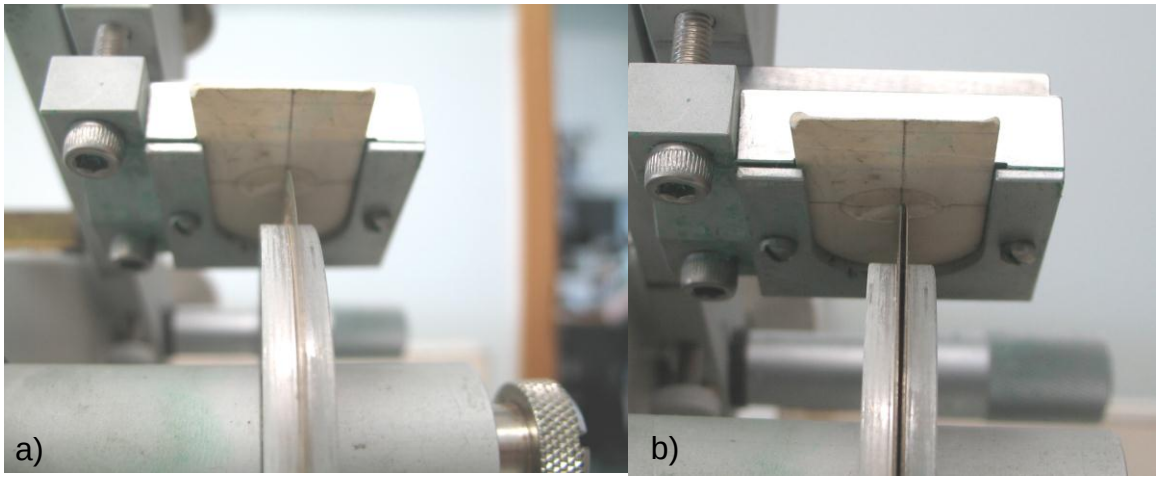

Figure 20. It is important to line up the saw blades along the sectioning line drawn on the tag in order to cut through the nucleus of the otolith. a) The single blade is lined up right on the cross-hairs. b) Double blades are lined up to straddle the cross-hairs.

2.2.1.3 Sectioning otoliths not encased in epoxy

Bisecting is the simplest and fastest machine method to cut an otolith in half through the nucleus using one saw blade (Fig. 21a). Both large and small otoliths can be cut with this method to produce a smooth section surface and a standard plane for measurements. The SCL uses the burnt otolith section method for most production ageing of otoliths. But, when otoliths cannot be broken manually with fingers or clippers because they have been broken off centre or due to their size, thickness or shape, the saw is used to make a cut through the centre of the nucleus. In many cases, the otoliths do not need to be encased in epoxy. It is possible to cut a thin section from an otolith that is not epoxied using two blades (Fig. 21b), however this can pose challenges as the plasticine may not hold the otolith firmly enough to avoid fracturing the section. Note: the SCL prefers otoliths broken by hand for the burnt method as the fractured surface usually burns with better clarity than a polished surface produced by a saw cut. The following preparations and procedures pertain to bisecting otoliths that have not been encased in epoxies or glue.

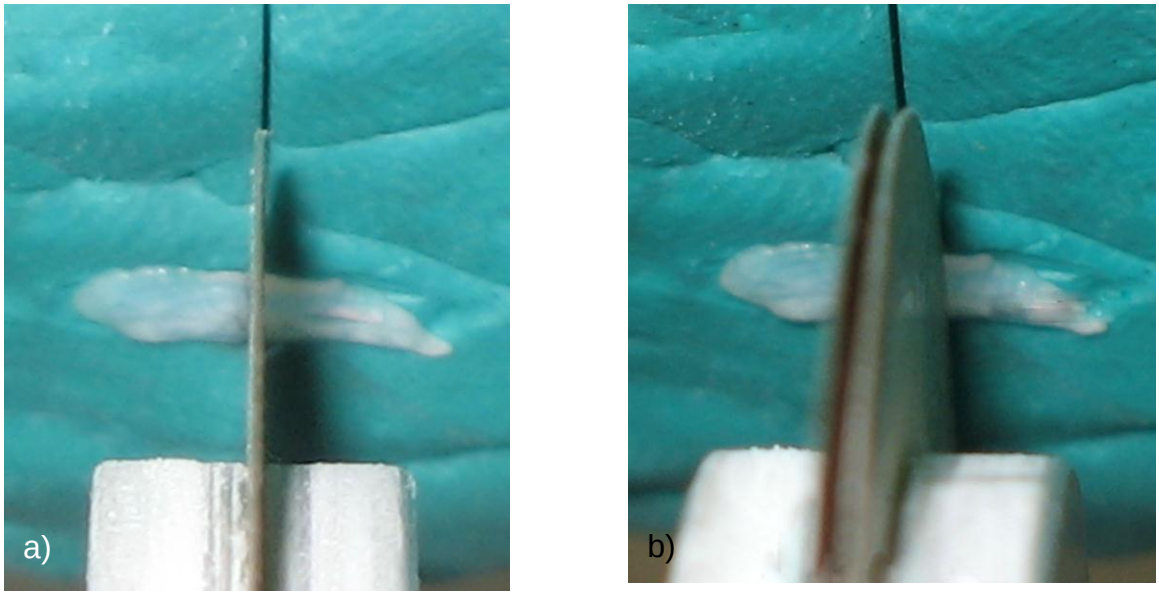

Figure 21. Non-epoxied otoliths can be sectioned without epoxy when pressed into the plasticine on the saw chuck. a) Illustrates how an otolith can be bisected by cutting with one blade. b) Shows how a thin section can be cut using two blades.

2.2.1.3.1 Equipment & Materials

- low-speed saw (e.g. Buehler® Isomet™)
- water
- plasticine
- forceps
- personal protection equipment: eye protection

2.2.1.3.2 Preparation

Preparation of the Buehler® saw is the same as when sectioning epoxied otoliths using one or two blades with plasticine applied to the chuck. The only difference is that the otoliths need no preparation for this method and are cut without the support of epoxy or glue.

2.2.1.3.3 Procedure

Follow the same procedures for cutting thin cross-sections from epoxied otoliths. Water and all traces of plasticine need to be wiped from the otolith cross-section surface if they are to be burnt for production ageing. Be sure that the plasticine is fairly firm to maintain a good grip on the otolith to keep it from moving during the cutting process.

2.2.2 High-speed saw method

Historically, the SCL used a modified Bronwill® TSM Universal Model 77 bone sectioning machine (Fig. 22) to section larger otoliths from species like Pacific hake (*Merluccius productus*) and rockfish (*Sebastes*) for production ageing before changing over to the burnt otolith section method. It is generally not recommended to use this saw for smaller or fragile otoliths or when very thin sections are required. The combination of water pressure, speed and power of the saw rotation in combination with blade width does not facilitate production of very thin sections. The SCL generally cuts otolith sections, with this machine, to a minimum of about 1mm in thickness. Any thinner and the section, or portions of the section, tend to pop out of the epoxy when being cut.

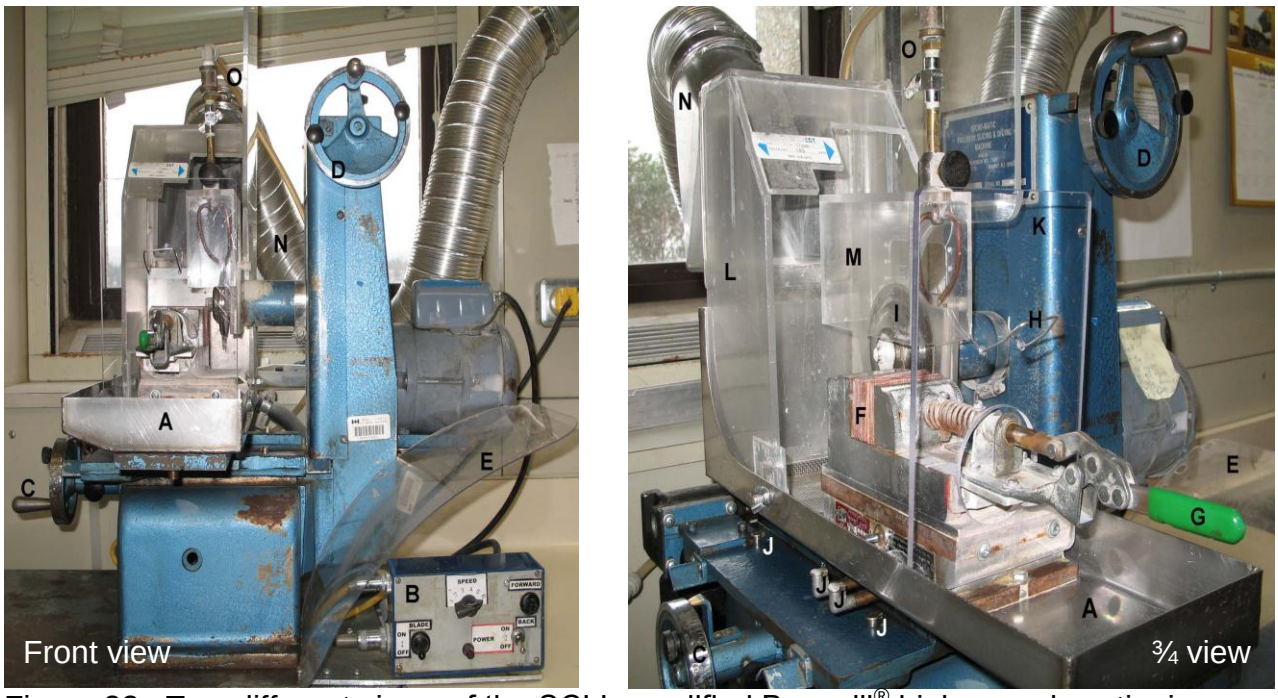

Figure 22. Two different views of the SCL's modified Bronwill® high-speed sectioning machine with parts labelled. A-bed reservoir, B-control panel, C-cross-feed handle, D-control wheel for blade height, E-flexible plastic water shield, F-chuck, G-green chuck lever, H-wire safety catch, I-saw blade, J-oil cups, K-marguard® face shield, L-plexiglass® hood, M-plexiglass® blade shield, N-exhaust system, O-water cooling system.

Water is delivered to the saw blade to reduce cutting friction. It is important to maintain adequate water pressure when cutting. Insufficient water can affect section quality, damage blades and produce a burning smell when sectioning. The cutting angle is also very important. A 45° angle to the horizontal reduces the cutting surface, minimizing friction so the blade cuts rather than pushes through the otolith. Pushing leads to oblique sections with reduced pattern clarity compromising the ability to produce accurate ages. Approved ear protection

must be used by everyone in the room when this machine is in operation.

To improve operational efficiency and safety the SCL has made a number of modifications to the original Bronwill® sectioning machine:

- Removed the oil reservoir and attached hoses to deliver tap water to cool the blade and capture fin and epoxy dust.
- Attached a customized vacuum system to a large Plexiglas® hood which removes air or water-bound particles dispersed by the cutting wheel.
- Inserted a clear, protective face shield of Marguard® to protect eyes from debris.
- Removed the control panel from the machine and placed it at table top level for ergonomic ease and protected it against water splash with a plastic shield.
- Added a wire safety-catch to the lever that clamps the epoxied otolith into the chuck to keep it from springing back at the operator's face.

2.2.2.1 Equipment & Materials

- high-speed sectioning machine (e.g. Bronwill® TSM Universal Model 77 bone sectioning machine)
- diamond saw blade (e.g. Norton® Diamond saw blade 4" X 0.015" X 0.500" 1/8" return)
- cloth net material and metal screen
- frosted glass slides (2.6cm X 17.6cm)
- fine permanent marker
- slide drying boards & rack
- 19 – 20cm forceps
- compound scope
- coin envelopes with epoxied otoliths
- health & safety: refer to sectioning machine modifications list
- personal protection equipment: lab coat, approved ear protection (e.g. earmuffs #LM-77 EN 352-1:1993 CE or earplugs), lab coat, fine particulate dust mask if dry sectioning non-epoxied fins (e.g. 3M 8210 Respirator N95 Particulate)

2.2.2.2 Preparation

1. Label slides using a fine permanent marker. Record all sample information on the first slide, such as: species abbreviation (e.g. Sablefish = SB) and fish number, species name and first and last fish number in sample, vessel name, area and date caught. The fish ID number must be written at the top of all slides so the number can be seen when the slides are stored in the slide box. All subsequent slides need only be labeled with species and fish number (e.g. SB1, SB2…) (Fig. 15).

2. Become familiar with the features and accessories of the Bronwill® machine. Locate all switches on the control panel (Fig. 22) to understand their use.
3. Position net material in bottom of carriage bed to prevent sections from clogging or going down the drain (Fig. 23).

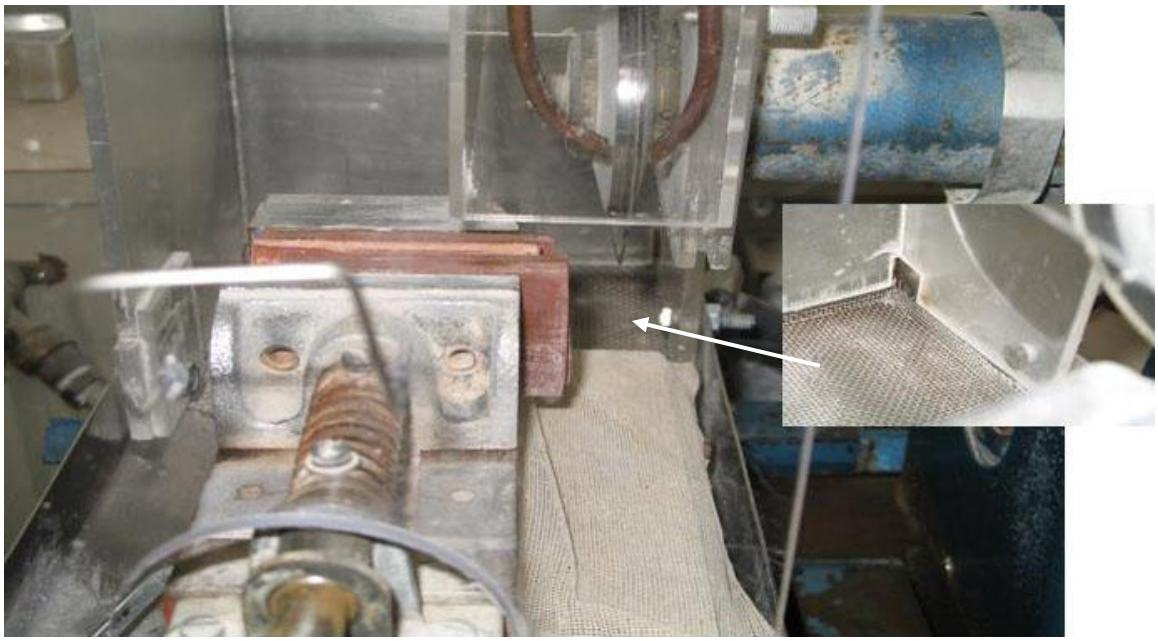

Figure 23. Close up of high-speed saw showing the net material and metal screen (inset) needed to prevent sections from being sucked into the bed reservoir drain hole at the back corner of the bed reservoir.

2.2.2.3 Procedure

1. Turn the fume hood on to high to activate the sectioning machine exhaust system.
2. Turn water source to machine on with enough flow to lubricate the saw blade when operating. Periodically check to make sure that the water is draining effectively from the bed reservoir and the drain is not blocked to prevent overflow.
3. Position the carriage bed horizontally with the cross-feed handle (left or right) to allow placement of the epoxied otolith into the chuck.
4. Remove the epoxied otolith from its envelope and place in the chuck by its epoxy tail with the distal otolith surface facing the operator. The embedded otolith should extend out from the chuck about 2.0-2.5cm to start with. Align the otolith perpendicular to the blade and adjust it to a position where the first cut will be close to the center of the nucleus but slightly to the right. Take into account the saw width if making multiple sections. Close the green vice-lever and engage the wire safety catch to prevent it springing back towards the operator's face. Cut a minimum of two sections.

5. Adjust the height of the saw blade by using the control wheel for height. Move the blade to a level where it is behind and near the top of the epoxied otolith. The blade must approach and make contact with the embedded otolith at a 45° angle from the horizontal so that the blade will effectively cut through the epoxied otolith.
6. Turn the sectioning machine and blade on.
7. Turn the carriage bed switch to 'Forward'. This will move the otolith toward the blade to make the first cut through the otolith until the right side end of the epoxied otolith falls into the carriage bed.
8. Turn the blade off and use forceps to retrieve the epoxy stub and place it back in the correctly numbered envelope.
9. Turn the carriage bed switch to 'Back' until the otolith is in position behind the blade again.
10. Use the cross-feed handle to move the bed horizontally to the right to position for the second cut. Take great care that repositioning the bed/blade for multiple cuts does not lead to the flanges or blade striking the chuck. This will cause damage.
11. Turn on the blade and make the 2nd cut. The section will fall onto the net covered bed floor.
12. Turn blade off and use forceps to remove the section and place it on the pre-numbered glass slide with the '2nd cut side' facing up.
13. Cut a total of 2 to 4 sections.
14. Place each section on the slide to the right of the previous one, with the last cut side up, orienting all sections the same, in sequence, start to finish – left to right (Fig. 18).
15. Place the slide with the sections on a drying board in the rack (Fig.19).
16. Remove the remaining portion of the epoxied otolith from the chuck and return it to the envelope.
17. Follow the above procedure for all otoliths in the sample.
18. Allow the sections to dry overnight before applying the liquid cover slip (Flo-texx®).

2.3. MOUNTING OTOLITH SECTIONS ONTO GLASS SLIDES

After the otolith thin cross-sections have dried overnight, a liquid cover slip (e.g. Flo-texx®) can be applied. The cover slip clarifies the growth pattern for age determination, preserves the sections and secures them to the glass slide. Thermal plastic cement/glue can also be used to fix sections to the slide if they are to be ground or polished or are not to be permanently mounted on slides. Application of Flo-texx® must be done inside an approved exhaust system (fume hood) to remove toxic fumes and personal safety equipment must be worn to avoid contact with skin and clothing.

2.3.1 Equipment & Materials

- probe
- liquid cover slip (e.g. Flo-texx®)
- health & safety: fume hood
- personal protection equipment: lab coat, disposable latex or nitrile gloves

2.3.2 Preparation

1. Move the slide rack with drying boards into the fume hood and turn the exhaust system on to high.
2. Remove the first board of slides from the drying rack and place inside the fume hood at a convenient distance for working. Close fume hood window to the recommended level.

2.3.3 Procedure

1. Starting with the first slide, use fingers to gently loosen the otolith sections from the glass. This encourages the liquid cover slip to flow under and attach them securely.
2. Place the sections close together oriented all the same way on the slide so that when aged the growth pattern can be traced from one section to the next.
3. To ensure that the glass slide will fit into slide box slots properly position the first section about 0.5cm to the right of the frosted label and the last section no closer than 0.5cm away from the right end of the slide. Sections should not project beyond the top or bottom of the slide.
4. Slowly drip the cover slip over each section on the slide, working from one end of the slide to the other. Ensure that the entire otolith section surface is covered on both sides. Do not allow excess to flow far beyond the sections or off the glass slide.
5. Immediately check each section for air bubbles that may form underneath or on top of the otolith section as it will obscure the growth pattern making it difficult to age. Use a probe to break the bubbles.

6. After completing the above for all slides on the drying board, return it to the drying rack.
7. Follow the above procedure for each board of slides in the sample, leaving them in the fume hood until the cover slip is completely hard. A thumbnail should not be able to dent the Flo-texx® if properly hardened.
8. Place slides into a slide box.
9. Label the outside of slide box with: species name, first & last fish number in sample, vessel name, area and date caught.

2.4 MAINTENANCE AND CARE OF SECTIONING MACHINES

2.4.1 Buehler® Isomet™ machine

The Buehler® machine requires regular maintenance and care to ensure it functions properly and in the long term. The sample basket and lubricant pan should be emptied and cleaned after each use. The basket will corrode if left in water for long periods. The diamond studded saw blades are very delicate and susceptible to damage, especially if too much force is used during sectioning. Avoid harm to the blade by only using the amount of weight needed for smooth cutting and engage the slide lock (added by SCL) to prevent the support arm from falling onto the blade when not in use. New blades must be cleaned with a dressing stick (Fig. 14b) 7-8 times before first use to remove metal matrix and to expose the abrasive grains of the blade for free cutting action. The stick should be attached to the chuck for dressing rather than pushed into the blade by hand. The blade needs to be dressed regularly after each day's use to remove epoxy and/or plasticine particles for efficient cutting.

2.4.2 Bronwill® TSM Universal Model 77 bone sectioning machine

It is important to carry out regular maintenance to keep equipment in good working order to avoid breakdowns that might otherwise cause delays in meeting deadlines. The following describes the maintenance specific to the Bronwill® sectioning machine used by the SCL.

1. Clean all surrounding Plexiglas® and Marguard® after completing a sample or after each day's use: detach and clean the main Plexiglas® hood, blade shield and the Marguard® face shield with a soft cloth or soft paper towel and warm soapy water.
2. Remove and wash the net material spread below and to the right of the chuck and the metal screen from inside the machine bed. Clean inside the machine bed and the chuck. Check that the drain pipe is clear.

After eight hours of machine use, add a few drops of oil to the 6 oil cups. Four are located on the left of the bed and two on the right side of the bed.

3.0 GLOSSARY

Accuracy – In terms of age determination, a measure of how close an age is to the true value.

Acellular – Non-skeletal bone formation.

Allometric growth – In terms of otoliths, the process where growth (rate, amount) varies along different growth axes.

Annual zones – Consists of one summer (opaque) zone and one winter (translucent – annulus) zone.

Bisect – Refers to cutting an otolith transversely in two through the nucleus.

Chuck - Mechanism/device on a machine used to mount/attach otoliths for sectioning.

Distal – Side of the otolith that is situated furthest from the body centre or point of attachment.

Dressing stick – A block of silicon/aluminum oxide used to dress (clean) diamond saw blades to remove the buildup of materials left behind from sectioning.

Epoxy bed or plug – A rectangle of epoxy formed to embed otoliths that facilitates the sectioning process.

Longitudinally – Refers to the longest axis of otoliths.

Nucleus –The region of an otolith where growth originates.

Oblique cut – This produces otolith cross-sections with unclear and ambiguous growth patterns that are difficult to identify and count. They are usually caused by incorrect positioning of the otolith when mounted in epoxy or onto the chuck for sectioning so that the cutting angle is shifted away from the preferred angle, i.e. perpendicular to the otolith's longest axis.

Proximal – Side of otolith that forms closest to the body centre.

Precision – Degree or measure of repeatability between two or more independently generated age estimates.

Production ageing – Regular (e.g. annual) age determination of large numbers of commercially important species for stock assessments and fisheries management.

Sagittae or sagittal otoliths – Usually the largest pair of otoliths (of three pairs) found in a boney fish's middle ear and most commonly used for age determination.

Skeletal bone – All bones in a fish's body other than otoliths.

Sulcus – Groove that forms on the proximal side of an otolith.

Technical errors – Errors introduced during the preparation and sectioning of otoliths that affect the quality of the section and hence the age data.

Transverse cross-section – Produced by cutting an otolith through the nucleus perpendicular to the longest (anterior to posterior) growth axis.

Viscosity/Viscous - Fluids that have a glutinous quality that will maintain shape or form rather than being runny.

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