Center for Quantitative Fisheries Ecology

Protocol

Preparation of Otolith Transverse Thin-Sections for Age Estimation of

Striped bass

Morone saxatilis

Contents

Equipment and Supplies

Item	Model/Source	Number/Use
Leica MZ 95 or Leica MZ12 Stereo-microscope with transmitted light source and polarizing filter		1
Buehler® IsoMet™ low-speed saw	Model number 11-1280-160	1
Flanges, steel	6.03 cm diameter	2
Spacer, steel	0.5 mm thickness, 6.03 cm diameter	1
Norton® Diamond Grinding Wheel	1A1 3 x 0.006 x 1/2" ME120928, M3D220-N75M99-1/8, UPC/Cup: 69014192342	2
Allen wrench	1/8 in.	1
25 gram weights	Buehler/1180S33	1-2
Water for IsoMet™lubricant tray		approx. 300ml, unfiltered water
Barnstead 1400 Thermolyne furnace	VWR 30605-022	1
Microscope slide [1 inch x 3 inches x 1.2 mm]	VWR 48318-0	1 per otolith plus 4-6 extras for "sectioning slide"
Microscope slide storage box	VWR 28511-012	1 per 100 otoliths
Aluminum slide tray	VWR 48467	1 per 20 otoliths
Porcelain Color Plate	VWR 53636-105	1 per 12 otoliths
Barnes glass eye dropper and dropper bottle	VWR 14216-246	1
Dissecting forceps	VWR 82027-398	1 fine point, 1 broad tip
Cordless precision engraver	General tools and instruments 505	1
Metal Spatula	General store brand	1
Flo-Texx® liquid cover slip	Lerner Laboratories	1 quart
Kimwipes® Delicate Task Wipers or VWR Light-Duty Tissue Wipers		1 box per work station for any spilled or Flo-Texx®
CQFE striped bass otolith storage box, cardboard		enclosed with labeled coin envelopes and microtubes containing individual fish's otoliths
Sharpie® Ultra Fine Point Permanent Marker		1

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Item	Model/Source	Number/Use
Hard-Part Processing Log		lists selected hard-parts to be processed, categorized alphabetically by species
Silicon baking tray	Fresh ware 24-cavity	1
West System®	105-B	3.7 liter
West System®	206-B	814 ml
Small paper Dixie® cup		3 oz.

Introduction

The following is a protocol for the preparation of sagittal otolith transverse cross-sections from striped bass (*Morone saxatilis*) for age determination. This protocol is to be used after the protocol for collecting biometric data and extracting whole sagittal otoliths. The protocol will first briefly introduce the structure of whole sagittal otoliths from striped bass, and then describe the detailed processes of mounting and sectioning the otoliths, and mounting and storing the completed transverse cross-sections for age determination.

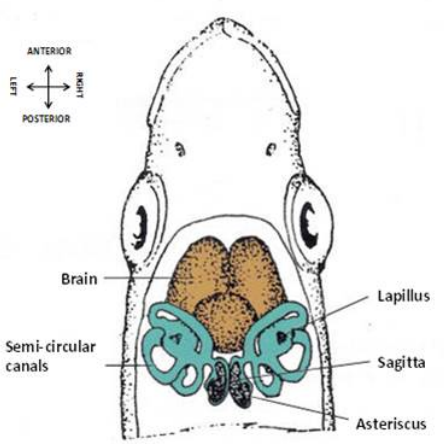

Figure 1: Position of otoliths within the vestibular apparatus of typical Teleost species, e.g., striped bass (Secor et al. 1992).

Structure of whole sagittal otoliths

Three pairs of otoliths (sagitta, lapillus, and asteriscus) are located within the vestibluar apparatus of typical Teleost species (Figure 1). They all play important roles in the sensory systems of these fishes for mechanoreception and maintenance of equilibrium in their environment. The sagitta is the largest of the three and used for ageing finfish.

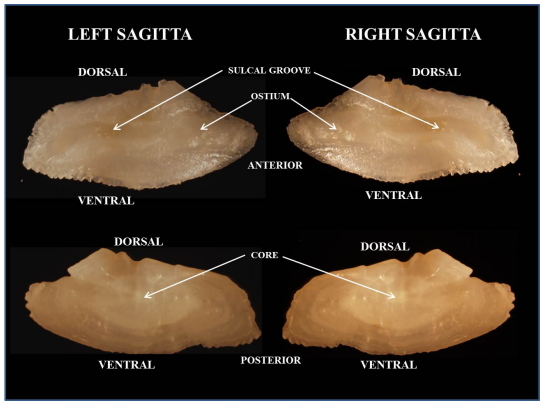

Figure 2: Extracted left and right sagittal otoliths of striped bass labeled to illustrate orientation and basic structure.

Striped bass have small otoliths relative to their body size. Unlike *Sciaenids*, their saggitae do not have a unique, tadpole-shaped sulcus acusticus (sulcus or sulcal groove), but, rather a sulcal groove that extends from the posterior edge to the anterior edge (Figure 2). For purposes of this protocol, the sagittal otoliths will be referred to simply as "otoliths".

As in all finfishes, striped bass otoliths are formed through biomineralization: specifically, the extracellular crystallization of calcium carbonate (primarily aragonite) onto an organic matrix composed of a keratin-like protein called "otolin" (Panfili et al. 2002).

Otolith formation begins early in the development of a fish, typically surrounding the hatch-date of the larvae. The initial structure that is mineralizied is called the primordium, or primordia, which fuses to form the otolith core. This core is the foundation on which all new otolith growth occurs. Concentric layers of protein and calcium carbonate matrix accrete outward from the core throughout the lifetime of the fish, resulting in a structure that is comparable to that of an onion.

Within the otolith matrix, aragonite is pre-

cipitated at varying rates throughout each year. Periods of slower growth in the fish, i.e., colder seasons of the year, are characterized by densely-packed precipitate. The core and opaque layers of the otolith, as visible through transmitted light, respresent such growth. Periods of faster growth in the fish, i.e., warmer seasons of the year, involve less-dense, compacted mineralization of the precipitate and are indicated by translucent layers of the matrix when viewed in transmitted light. The collection of successive opaque and translucent layers within the otolith can be made fully visible when a transverse cross-section (hereafter referred to as "thin-section") is removed from the core region (Figure 3) and viewed through a stereomicroscope.

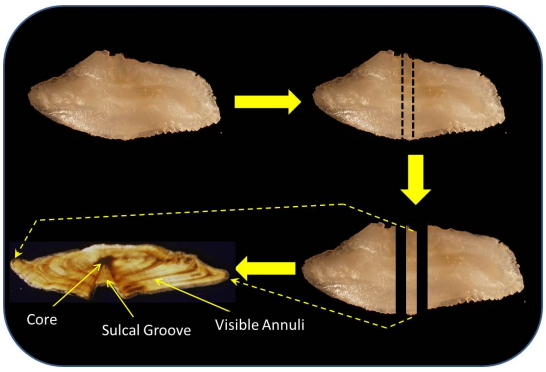

Figure 3: Striped bass otolith, thin-section removal, and visible annuli within the section under transmitted light.

Each of the opaque and translucent layers within the otolith constitutes an annulus, which occurs once per year. For the purposes of age determination, only the opaque layers encircling the core are called annuli; they are counted from the core towards the outer-edge of the otolith thin-section.

Preparation for sectioning

Begin by turning on the Barnstead/Thermolyne 1400 Small Benchtop Muffle Furnace (hereafter referred to as "oven"). The temperature setting should be set to 400 °C (Figure 4).

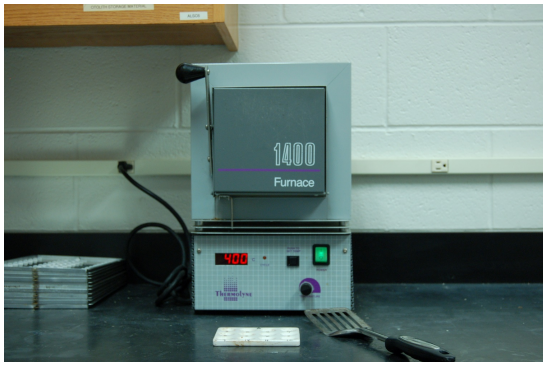

Figure 4: Barnstead/Thermolyne 1400 Small Benchtop Muffle Furnace.

To set the temperature:

1. Depress the black button that reads "Push to Set Temp" above it;
2. Turn the "Tempurature" knob until the display reads 400 °C;
3. Release the black button.

While the oven is warming, find the species and hard-part that has been selected for processing from the Hard-Part Processing Log. Search for the coin envelope with the selected Age and Growth ID number within the striped bass otolith storage box. The AGIDs are found on the lower right-hand corner of each envelope.

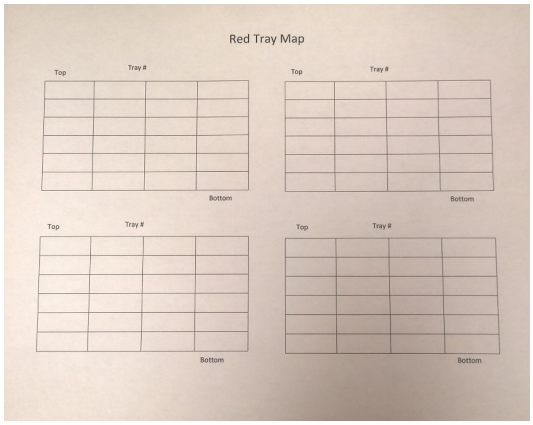

Figure 5: Tray map.

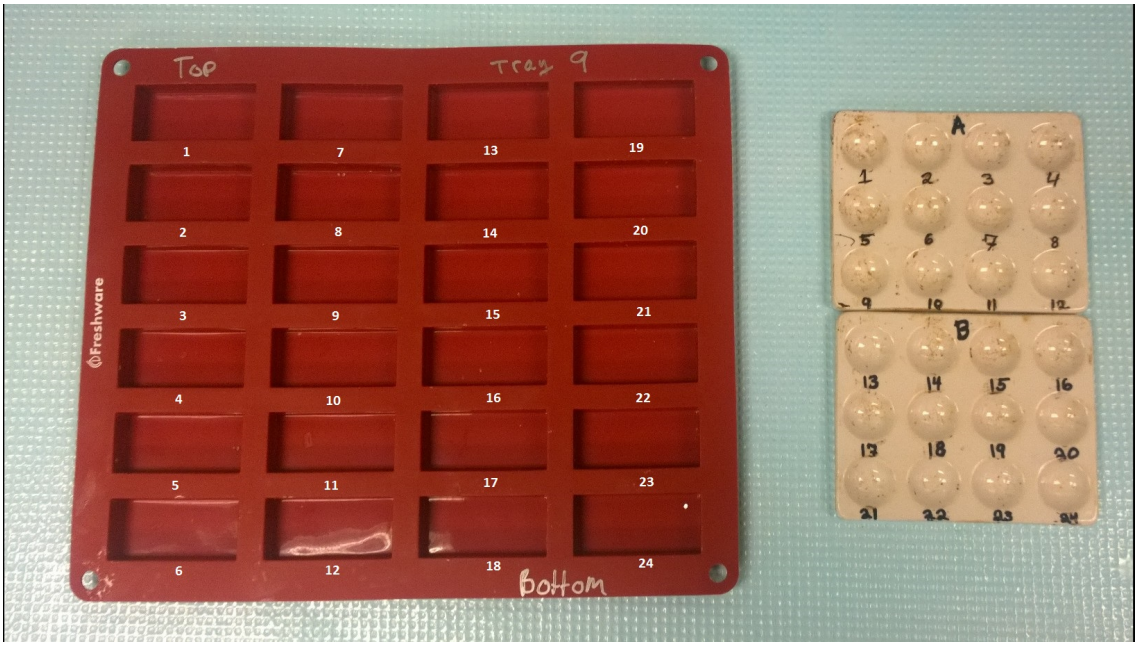

Figure 6: Silicon tray and ceramic plate.

Then, select the tray map (Figure 5) that corresponds with the silicone tray that will be used to set the otoliths. Record the tray number as wells as the AGID of the otolith starting with the top left cavity going down each column and ending with the bottom right cavity.

Once the map is appropriately labeled, remove one otolith from the microtube in the coin envelope and place in the cavity that corresponds to the AGID on the map. Do this until the silicone tray is full.

Transfer the unbaked otoliths one at a time to the CoorsTek® Spot Plate (hereafter "ceramic plate"). Be sure to move the otolith in cavity one to well one in the ceramic plate, continue with cavity two to well two, etc. (Figure 6). Once all of the wells in the ceramic plate have been filled, use the metal spatula to pick up the filled ceramic plate and place it inside the oven. Close the oven door and set the timer for two minutes. When the timer goes off, remove the ceramic plate with the metal spatula. Examine the otoliths' color. A light caramel color is ideal for aging. If the color is not caramel enough, put the ceramic plate back into the oven for 30 seconds. Sometimes it only takes one additional 30-second bake; sometimes it takes three to four additional bakes. There should not be any charring or large black spots on the otoliths, indicating the otoliths have been over-baked. In some situations the charring can be removed by scraping it with tweezers. When the light caramel color is achieved, transfer the baked otoliths from the ceramic plate to the silicone tray in the same order the otoliths were originally moved so that the AGID of the otolith matches the AGID on the map.

The otoliths are now ready to be embedded in West System® 2-part epoxy resin (hereafter "resin"), part 1 is the 105-B epoxy resin (hereafter "epoxy") and part 2 is the 206-B slow hardener (hereafter "hardener"). The resin is a 5:1 epoxy to hardener ratio. More specifically, to achieve the required ratio, the epoxy is in a 126.6 fl. oz. (3.7 liter) container and the hardener is in a 27.5 fl. oz. (814 ml) container (Figure

7). To mix the correct ratio you will need one pump of the epoxy and one pump of the hardener into a small paper cup (e.g. Dixie® or similar). Be sure to mix the epoxy and hardener well but stir slowly to minimize air bubbles in the mixture. Fill the silicone cavities until the resin is just above the top of the otolith, be sure to pop or move any air bubbles from around and underneath the otolith. Leave the resin to cure for 24 hours

Figure 7: Epoxy resin and hardener.

Once the resin is cured, label each block with the AGID in permanent marker and remove from the cavities. After removing from the cavities, use a dissecting microscope to mark the core of each otolith using a fine-tipped Sharpie® (Figure 8).

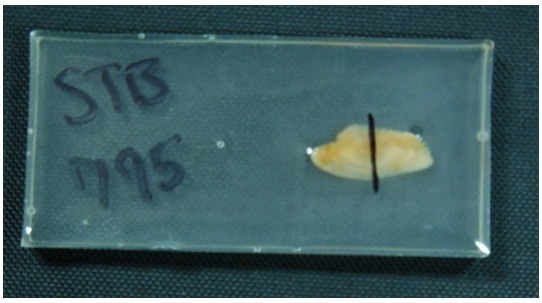

Figure 8: Otolith embedded in resin block with its core marked by Sharpie.

Sectioning otoliths

Before cutting the otolith, make sure that the Buehler® IsoMet™ low speed saw (hereafter IsoMet™ saw) is set-up correctly. From left to right on the drive-shaft there should be a shaft spacer and slinger followed by an inner flange, a Norton® diamond blade (Figure 9), a 0.5 mm spacer, an additional Norton® diamond blade, an outer flange, and an end cap bushing. The previous items are fixed to the drive shaft by a hand-tightened thumb screw (Figure 10). The saw's lubricant pan should be filled with unfiltered water and the specimen basket should be in place (Figure 11).

Figure 9: Norton® Diamond Grinding Wheel.

Place the marked resin block into the chuck of the saw's support arm (Figure 12), and secure it using the allen wrench. Once the resin block is secured within the chuck, use the micrometer (Figure 13) to align the support arm and bring the cut line marked on the otolith into position between the two Norton® Diamond Grinding Wheels (hereafter "blades"). The line should run completely parallel to both blades, and fall directly within the 0.5 mm space between them (Figure 14).

When all of the IsoMet™ saw specifica-

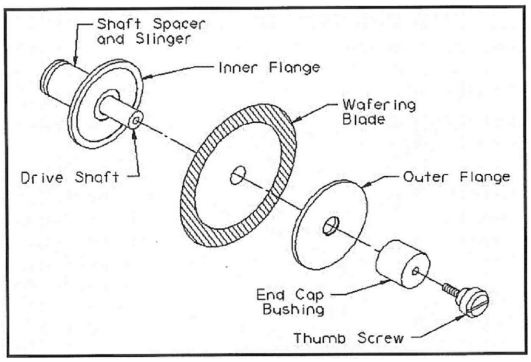

Figure 10: IsoMet^{TM} low speed saw blade installation diagram, showing the order flange and Norton® Diamond Grinding Wheel placement on the drive shaft. Note, that in our procedure we use a 0.5 mm spacer between two Norton® Diamond Grinding Wheels (modified from Buehler® IsoMet^{TM} Low Speed Saw Manual).

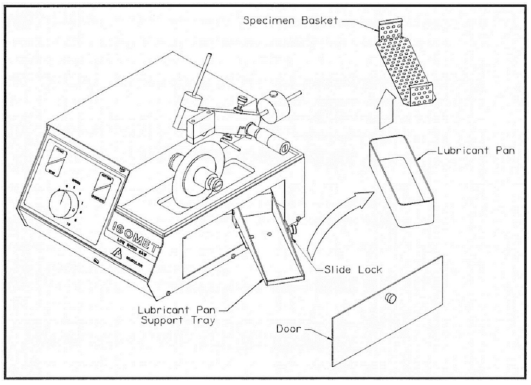

Figure 11: IsoMet^{TM} saw lubrication pan diagram (Buehler® IsoMet^{TM} Low Speed Saw Manual).

tions have been met, it is safe to start sectioning the otolith. Before moving the support arm and placing the resin block on the blades, start the saw at a speed of 3 or 4. Once the blades have begun spinning, gently move the support arm downward onto the blades, bringing the resin block into contact with the blades. Allow them to cut at this speed for several seconds. Once the blades have established a groove, bring the saw speed up to 7 or 8. The blades should now be close to cutting through the otolith, separating the thin-section from the matrix. It should take 3 to 5 minutes to com-

Figure 12: Marked resin with otolith embedded is mounted in the chuck of the saw arm.

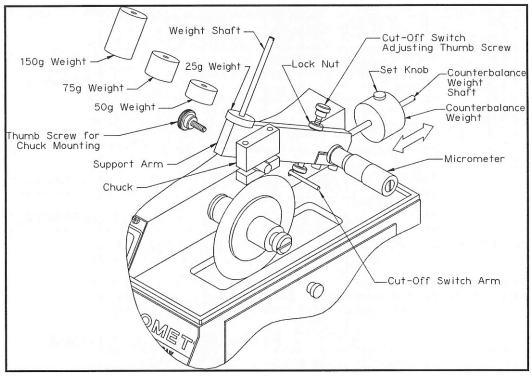

Figure 13: IsoMet^{TM} saw weight-balanced diagram (Buehler® IsoMet^{TM} Low Speed Saw Manual).

plete the cut. In some cases the cut will take longer, maybe over 10 minutes.

The cutting time can be decreased by adding up to 50 grams of weight to the weight shaft at any point during the sectioning process. Increasing the saw speed will also decrease the amount of time per otolith. Resin is dense and can withstand higher speeds than the 7 or 8 setting.

Note that additional weight and speed will increase the risk of damaging the saw blades and/or the otolith sections. Technicians must use their discretion, based on personal experience, in sectioning otoliths to maintain quality and safety.

While the blades are still cutting the resin block, using a cordless precision engraver,

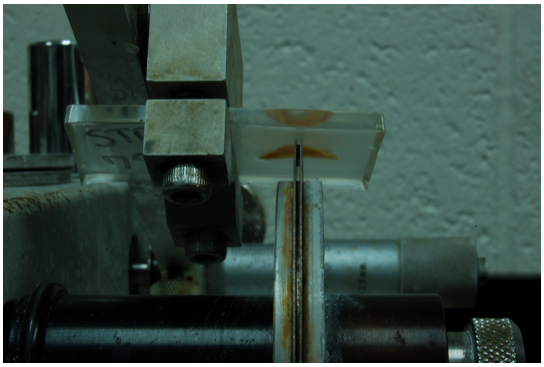

Figure 14: The black Sharpie® mark is lined up right between 2 blades.

engrave a clean microscope slide (hereafter referred to as "slide") on the left end with abbreviation name of striped bass (STB), AGID (e.g. 269), VMRC, and the year abbreviation (e.g. 09 for 2009). This slide will be used for mounting the thin-section being cut.

Monitor the cutting progress and the advancement of the blades through the resin and otolith. From the back of the resin block, you can view the otolith matrix becoming thinner as the blades near the back of the resin block. Once the blades cut through the entire resin block, stop the saw. Collect a large piece of resin remained in the chuck and a small piece of resin fallen in the lubricant water, and put them back in the coin envelope from which they came.

Very often, the thin section will stay between the blades. Remove the thumb screw and the outer flange from the drive shaft. Pull the two blades and spacer off of the drive shaft and lay them flat on your palm or on the work table. Separate the top blade from the spacer and the inner blade and search for the thin-section on the spacer side of the blades. If the thin-section is not between the blades, then it has fallen into the lubricant water. Pull out the lubricant pan, lift the specimen basket out of the water, search for the thin-section, and collect it using tweezers. Using a Kimwipe® gently dry the thin section off and place on the labeled slide.

Mounting otolith thin-section

Before permanently mounting the otolith thin-sections, look at them under the Leica stereomicroscope to make sure that the section must include the core. The sulcal groove should meet the core at a precise angle such that all annuli can be seen from the origin to the edge of the otolith, and sectioned sulcal groove has a "V" shape (Figure 15).

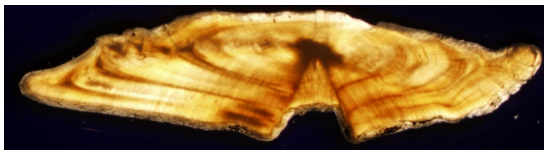

Figure 15: Completed transverse thin-section with a "V" shape of sectioned sulcal groove (positioned as the opening of the "V" downward on the slide), indicating that the cut went through the core.

If the sulcal groove and the core do not come together to form a point, and the sectioned sulcal groove looks like a "tornado" shape, then, the cut was placed too far from the ostium (Figure 16).

If the sulcal groove does not come together to form a point, and the sectioned sulcal groove looks like a "dome" shape, then, the cut was placed too close to the ostium (Figure 17).

A section with a correctly executed cut should have no chips or other imperfections that eliminate or obscure views of the core, sulcal groove, or annuli. Sections not meeting these specifications must be re-cut. The second section may be obtained from either of the two halves of the first otolith (depending on where the first section misses the core), but the second otolith may also

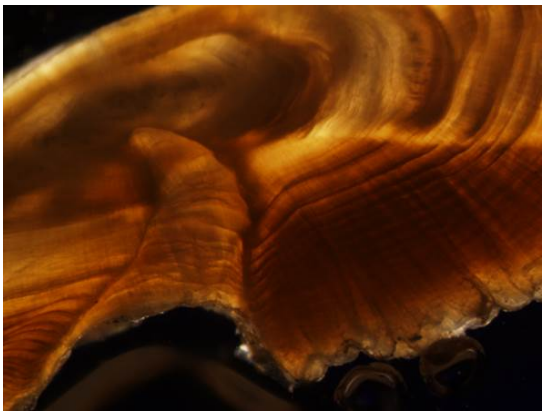

Figure 16: A transverse thin-section with a "tornado" shape of sectioned sulcal groove (showing the opening down here), indicating that the cut misses the core and is far away from the ostium.

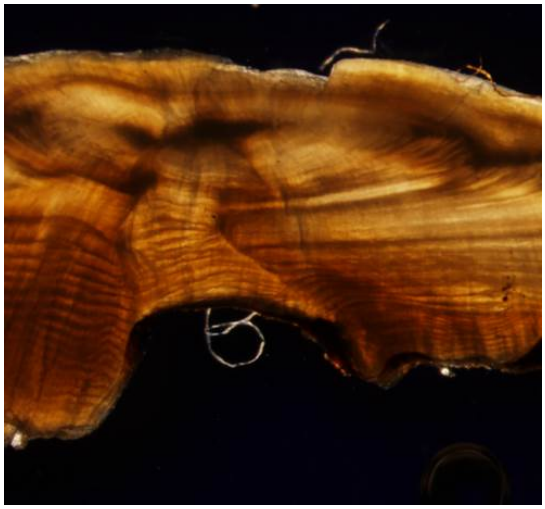

Figure 17: A transverse thin-section with a "dome" shape of sectioned sulcal groove, indicating that the cut misses the core and is too close to the ostium.

be used if necessary. When you have verified the section's quality, place the best surface of the thin-section facing upwards and ensure the section is siting flat on the slide engraved previously. Make sure that the opening of the "V" shape is positioned facing the long edge of the slide while the engraved end of the slide is facing to the left of reader (Figure 18).

When the section is clean and dry, protect it with Flo-Texx®, a liquid cover slip. Use an eyedropper to put a small amount of Flo-Texx® over the section and spread it in a circular motion. Eliminate bubbles within the Flo-Texx® by popping them or moving them away from the otolith using tweezers. The Flo-Texx® is used as the mounting medium to both protect the thin-section and increase its clarity. Place the completed slides on an aluminum slide tray and allow the Flo-Texx® to air-dry on the sections for several hours (until solidified).

Storing otolith thin-sections

Using a fine point black Sharpie®, write the abbreviation name of striped bass (STB) and fish ID (629) in the upper right-hand corner (opposite to the engraved end) on the long side of the slide edge (This step may be done while engraving the slide). Figure 18 shows the final otolith thin-section slide correctly labeled with abbreviation of species name, fish ID, VMRC, and collection year. Store the slides in a labeled slide box (Figure 19) with the black Sharpie® AGID facing upwards for easy identification during age determination.

Figure 18: Completed striped bass otolith thin-section, labeled appropriately with species code and AGID.

Figure 19: Otolith slide storage box, labeled for striped bass.

Literature Cited

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Secor, D.H., Dean, J.M., and Laban, E.H. 1992. Otolith removal and preparation for microstructural examination. In Otolith microstructure examination and analysis (Stevenson, D.K. and Campana, S.E., ed.).

Buehler® IsoMet™ Low Speed Saw Manual. Model MA111280_16.1 as of 6/8/2009.

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