Standard Operative Procedure for Embedding and Sectioning Blueline Tilefish (*Caulolatilus microps*)

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SEDAR50-DW08

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Standard Operating Procedure for Embedding and Sectioning Blueline Tilefish (*Caulolatilus microps*)

17 June 2016

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The purpose of this write-up is to describe the sectioning process of Blueline tilefish (BLT) sagittal otoliths.

Overview

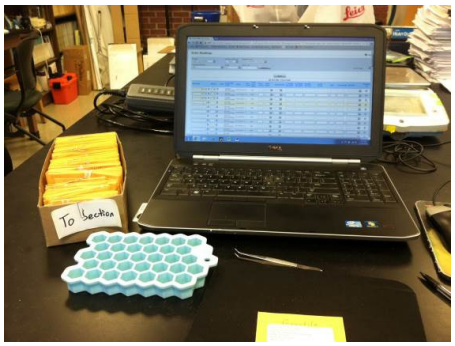
The best processing technique for BLT otoliths was embedding the sagittal otoliths in resin due to their frail nature from the deep sulcal groove. Prior to embedding in a two-part epoxy resin (Beuhler), the core area on each otolith was marked for ease when sectioning. Once the epoxy was hardened, excess epoxy material from the mold was trimmed off using the Hillquist machine or a dremel tool, and the remaining mold containing the otolith was affixed to a slide for sectioning. A set of three thin (~0.4-0.5 mm), serial sections was taken around the core of the otolith on a Isomet saw and then adhered on a slide in the order the sections were made. The middle section should contain the core. Once the sections were on the slide, they were polished on the Hillquist machine to a final section of 0.32 mm thickness. This further polishing after sectioning aids in clarifying annuli and the dense core of the BLT otolith for aging. A liquid cover slip, Gurr (aka DePex Mounting Medium), was applied to the sections and allowed to set up overnight before reading.

Preparation of embedding molds

1. Prior to labeling samples, the bottom half of epoxy molds need to be poured and allowed to harden.
    a. See appendix for supplies, instructions and amounts.

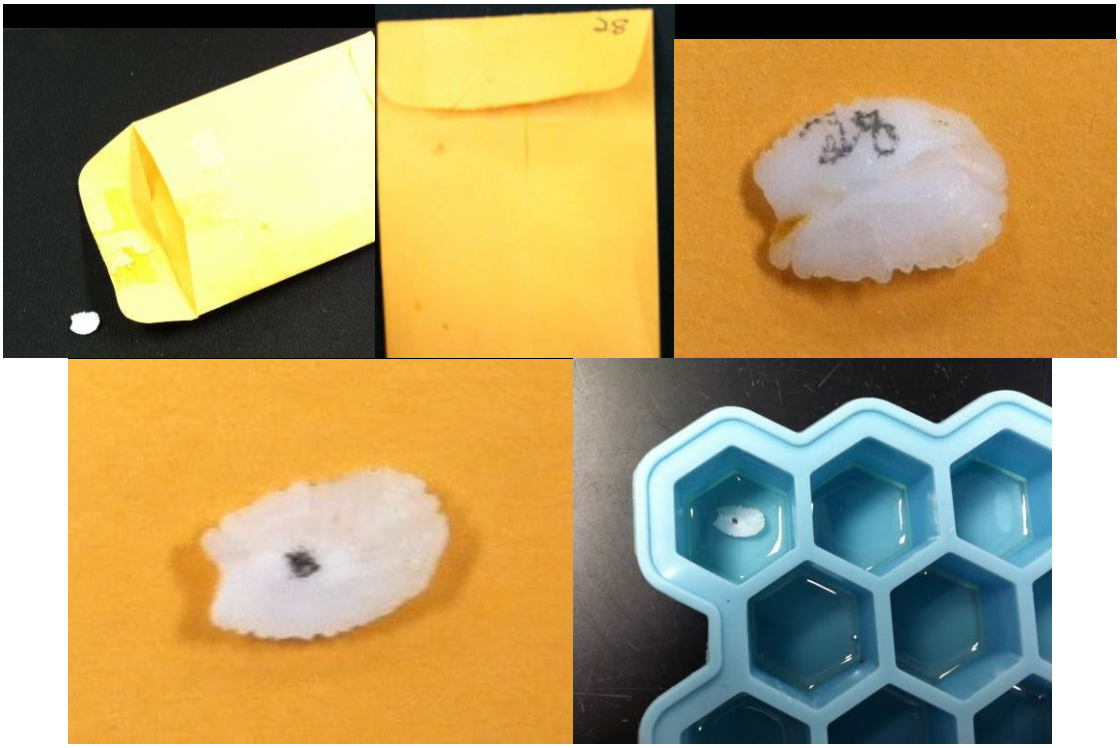
Labeling samples

2. Put sample envelopes in order based on year and interview #.



3. Each slide box should contain 90 slides (due to label sheets), each with 1 sample per slide

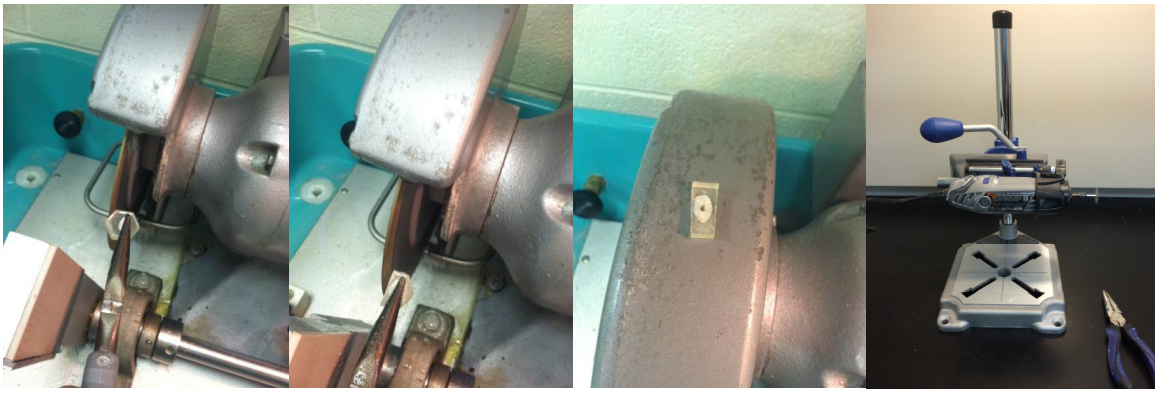
4. Remove otolith sample from envelope, mark envelope and back of otolith with slide # (again 1-90), mark core area, and place into mold.



5. Once all samples have been labeled and otolith placed in half-poured, cured molds, finish molds by covering otolith samples with more epoxy as described in appendix and allow to dry overnight. Once cured, pop out all samples and prepare to trim on Hillquist.

Trimming

6. The excess hardened epoxy mold needs to be trimmed in order for the saw blade on the Isomet to cut completely through the sample. This can be completed on a Hillquist machine (or with the use of a Dremel rotary tool with Diamond wheel attachment) and needle nose pliers. With pliers in hand, hold middle of sample, exposing one edge and apply to cutting wheel until complete. Switch sides of samples so that other side is exposed and repeat trimming process so that all that is left is a rectangular shaped mold. Repeat with until all samples have been trimmed.

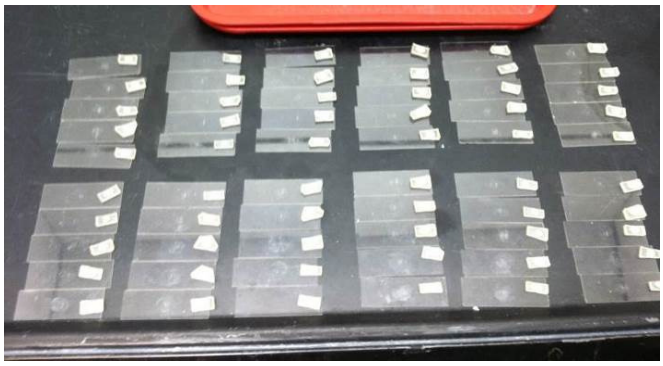


7. Put all samples in order from 1-90 on a tray. The sample number written on the proximal side of the otolith will be visible through the epoxy.

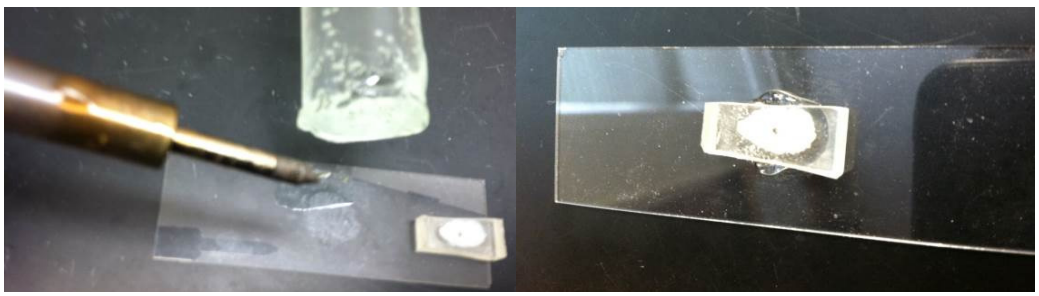
Sectioning

8. Supplies needed for fixing embedded samples to slides include: soldering iron, crystal bond (thermal glue), slides, and samples. Work needs to be completed in fume hood due to fumes when crystal bond is melted.

9. Line up all 90 slides and place samples on right edge of slide so that you can complete all samples in one sitting.



10. Once the soldering iron is hot, touch crystal bond to soldering tip over the middle of the slide and to left of sample. Allow 3-4 drips of crystal bond to drop on slide, lay crystal bond down, place embedded sample on liquid drops and push down, trying to line the otolith up parallel to bottom of slide. Be careful not to touch hot crystal bond as you will burn yourself. The crystal bond will cool rapidly, so placing and aligning the embedded otolith will need to be done quickly. Repeat for all samples



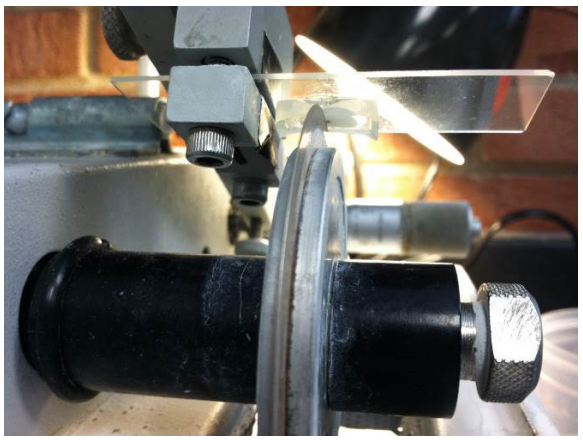
11. Line up all samples (IN ORDER) on tray for sectioning.

12. Sections are taken using an Isomet low-speed saw **at a speed of 9 and with the 75 and 25g weights (100 g total weight)**. **A single, 3 inch blade is used to section**. In order to ensure we have the core area and due to the difficulty in aging blueline tilefish, we take 3 serial sections. Methods for insuring consistency are as follows:

    a. Make sure the otolith is lined up correctly to get a true dorsal-ventral transverse cut of the otolith, adjusting the angle of the slide if necessary.

    b. With the saw off, line up the blade on the left side of the mark on the core area, laying it to rest on the blade.

    c. Noting which number on the saw micrometer arm, after lifting the sample off the blade, turn the micrometer knob one complete turn, twisting the knob counter clockwise, or toward you.



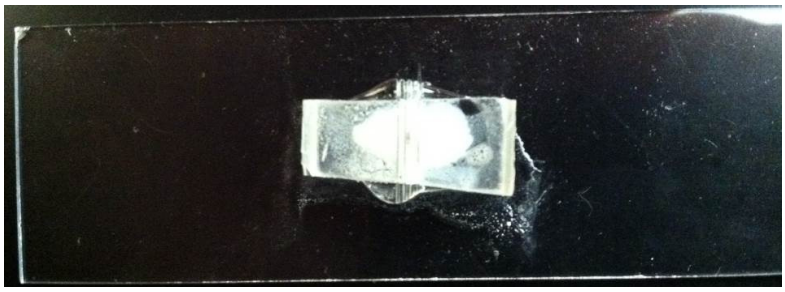
    d. Lower the sample back on the blade, seeing if the turn has captured a majority of the marked core area. If it doesn't, adjust until comfortable with the two cuts.

    e. Lifting the sample off the blade again, do one more rotation, again twisting the knob counterclockwise. This will be the location of the first cut, the furthest to the right.

    f. Turn on the saw and lower the sample to begin cutting. Once the sample is finished cutting, (listen for the changes in pitch - it usually goes from low to high, indicating

that the glass slide is being cut), lift the sample off of the blade, leaving the saw running, turn the knob one full rotation clockwise, or away from you.

g. Lower the sample, repeating the cutting process 3 more times until a total of 4 cuts are made on the sample. Remove sample from chuck. Sample should look like this.

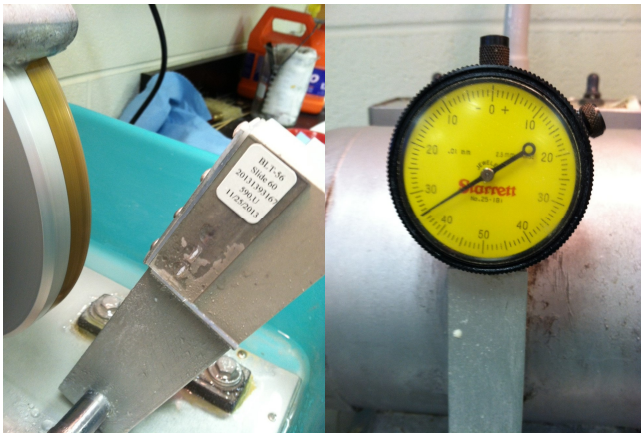


13. Using forceps, remove samples from left to right and place on slide preloaded with dots of crystal bond in the same fashion, keeping sectioned samples in order.

14. Place slide on hot plate, allowing it to warm up before removing and, again using forceps, apply slight pressure on each section to "seat" the section and ensuring there are no air bubbles under the sample. Apply slide label to slide.

Polishing and Gurr

15. Again using the Hillquist, but this time on the grinding wheel side, polish the sections down to 0.32 mm, or 35 on the gauge.



16. After polished, each sectioned sample needs a liquid coverslip, Gurr, applied to it prior to reading. Line all samples on trays. Using a 45 degree tipped probe, apply Gurr by rubbing it on each section until all samples are completed. Allow to dry overnight.

<u>Samples are now ready for age readings</u>

Appendix: Mixing and Pouring molds

We found that the typical bullet molds were too small to hold some of the larger otoliths and would be too expensive to custom order. Instead, we used a silicon ice cube tray (http://goldas-inc.amazonwebstore.com/Outset-Ice-Cube-Tray-Hexagon-Small/M/B005A20RQ0.htm) and only filled with enough embedding material to cover the top of the otoliths. Any silicon style tray would work as long as the samples can easily be removed.

Notes:

A. Use Buehler EpoxiCure 2 Resin and Hardener at a ratio of 2.5g:1g, respectively.

B. Using the ice cube tray mold, 21 grams (total of both resin (15g) and hardener (6g) per mold is sufficient coverage for a bottom half. This works out to about 0.6g per individual mold.

    a. If embedding all three ice cube trays, a total of 63g: 45g resin and 18g hardener.

C. To cover the otoliths, 0.9 g per individual mold is enough. This works out to 22.5g resin and 9g hardener (31.5g total) per 34 samples

    a. If embedding all three ice cube trays, a total of 84g (due to 12 empty molds) with 60g Resin and 24g hardener.

Pouring molds:

1. Supplies needed: Silicon release spray, resin, hardener, trays, gloves, mixing cup, stirring rod, transfer pipette, paper towels, and scale. Make sure to complete in fume hood or well-ventilated area as process produces harmful vapors.

2. Spray ice cube trays with silicon release spray and wipe off excess spray. This process will extend the life of your trays.

3. Measure desired amount of resin then hardener into mixing cup (They can be mixed directly together, being very careful not to over pour, use of transfer pipette helped)

4. Mix well, stirring until there are no strings. Once bubbles are present and the mixture becomes clear, then the embedding medium is ready for transfer into the molds.

5. Once each mold has mixture in it, tip ice cube tray in all directions to ensure that the bottom is completely covered. Allow to dry overnight

6. Once otoliths have been placed in each mold, tops need to be completed (thus completely embedding otolith), repeat the process as above. Once mixture has been transferred, be sure to line up otoliths so they can be trimmed later. Allow to dry overnight.