Ageing Laboratory/Fisheries Management Division

Protocol

Preparation of Spines for Age Estimation

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Introduction

In the VMRC Ageing Lab we age Tautog *Tautoga onitis* using their pelvic fin spines (Hereafter referred to as "Spine"). Therefore, in this protocol we will use Tautog as an example to demontrate how to prepare spines for age estimation. The protocol will first briefly introduce the structure of Tautog fin spine, and then describe the processes of cleaning, sectioing, and storing them. Since some of the processes are the same as processing Tautog opercula and otoliths, the protocol will skip those processes, instead, refer them to their corresponding sections in Preparation of Opercula for Age Estimation and Preparation of Otolith Thin-Sections for Age Estimation posted at the Ageing Lab website.

Structure of Fin Spines

The fins are supported by bony structures: spines and soft rays. Fin spine is unsegmented, unbranched, more rigid and calcified compared to soft ray, and composed of dermal bone, consisting primarily of calcium phosphate (Kerr and Campana 2014). Unlike scales, spine is vascularized dermal bone with a canal at the center filled with a blood vessel (Figure 1). However, highly vascularized bone is often resorbed, starting at the center of spine and proceeding outward as the fish grows (Tzadik et al. 2017). This process can obsure or destroy annuli associated with early life history (Drew et al. 2006). Therefore, when we age Tautog pelvic fin spines following the procedure introduced by Elzey and Trull (2016), we continue to age Tautog otoliths and opercula at the VMRC Ageing Lab.

Prepare Spines for Sectioning

Clean spines

For convenience and efficiency Tautog opercula and pelvic fin spines collected from the same fish are stored in the same coin envelope and cleaned in the same way at the same time,

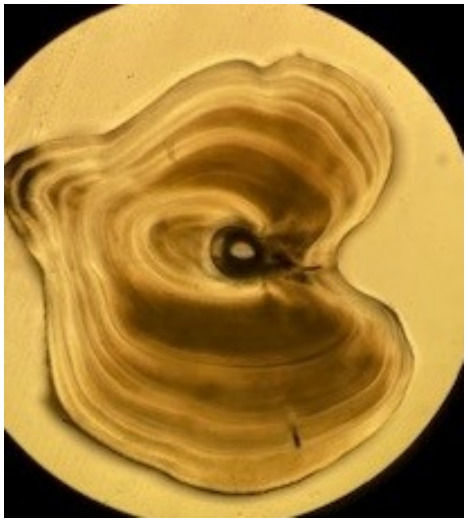


Figure 1: Tautog pelvic fin spine thin-section.

therefore, for the details on how to clean spines, users may go to Section "Clean Opercula" in Preparation of Opercula for Age Estimation posted at the Ageing Lab website.

Epoxy resin method

Make fish ID labels for epoxy resin blocks

Before putting a spine in a silicone well, make sure that the well is labeled with the fish ID of the spine. In the VMRC Ageing Lab we make the labels on a spreadsheet using an Excel macro which connects to the Lab data files. Print, cut, and put the labels in the silicone wells.

Put spines in silicone wells

We put spines in the silicone wells in the same way as we do otoliths, therefore, please refer to the method described in Section "Put otoliths in silicone wells" in Preparation of Otolith Thin-Sections for Age Estimation posted at the Ageing Lab website.

Embed spines with epoxy resin

We embed spines with epoxy resin in the same way as we do otoliths, therefore, please refer to the method described in Section "Embed otoliths with epoxy resin" in Prepara-

tion of Otolith Thin-Sections for Age Estimation posted at the Ageing Lab website (Figure 2).

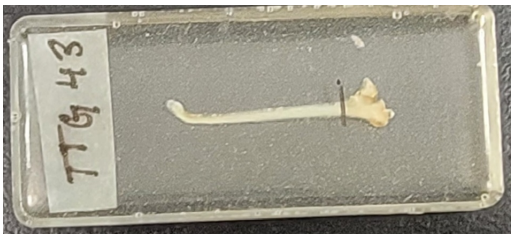


Figure 2: Tautog spine is embedded in a resin block for sectioning. Note: the black Sharpie marker is where the middle thin-section is located.

Section Spines

In general, sectioning a spine resin block is similar to sectioning otolith described in detail in Section "Section Otoliths" in Preparation of Otolith Thin-Sections for Age Estimation posted at the Ageing Lab website. However, we make one thin-section from one otolith using two blades and one spacer whereas we make three thin-sections from one spine using four blades and three spacers with the middle spacer aligned with the Shapie's marker on the resin block (Figure 3).

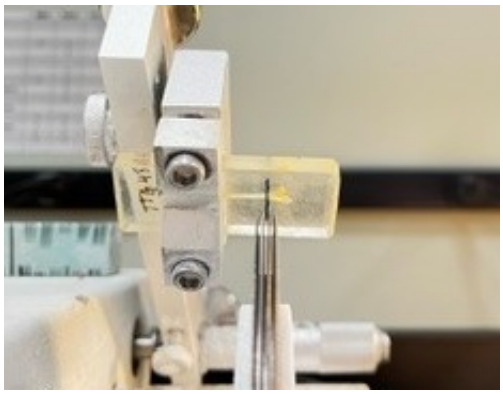


Figure 3: A Tautog spine resin block is placed on four blades, and the black mark on the block is aligned with the middle one of the three spacers. This setup will make three thin-sections of the spine at one time.

Mount Spine Thin-section on Slide

In general, mounting spine thin-section is similar to mounting otolith thin-section described in detail in Section "Mount Otolith Thin-section on Slide" in Preparation of Otolith Thin-Sections for Age Estimation posted at the Ageing Lab website. However, we mount three thin-sections from one spine on one slide (Figure 4).



Figure 4: Tautog spine slide with three thin-sections.

Store Spine Thin-section Slides

Store the slides in a slide box labeled with VMRC, collection year (2023), species name (TAUTOG), SPINES (indicating the box for spines), fish IDs, and number of boxes (Figure 5). Make sure the slide is positioned in the box with the black Sharpie® species name abbreviation and fish ID (Here is "TTG 20") facing upwards for easy identification during age determination.

References

Drew, K., D. J. Die, and F. Arocha
2006. Understanding vascularization in fin spines of white marlin (tetrapturus albidus). *Bulletin of Marine Science*, 79(3):847–852.

Elzey, S. P. and K. J. Trull
2016. Identification of a nonlethal method for aging tautog (tautoga onitis). *Fishery Bulletin*, 114(4).

Kerr, L. A. and S. E. Campana
2014. Chemical composition of fish hard



Figure 5: Spine thin-section slide storage box, labeled for Tautog.

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Tzadik, O. E., J. S. Curtis, J. E. Granneman, B. N. Kurth, T. J. Pusack, A. A. Wallace, D. J. Hollander, E. B. Peebles, and C. D. Stallings
2017. Chemical archives in fishes beyond otoliths: A review on the use of other body parts as chronological recorders of microchemical constituents for expanding interpretations of environmental, ecological, and life-history changes. *Limnology and Oceanography: Methods*, 15(3):238–263.

Equipment and Supplies

Item	Specification
I. Cleaning:	
Stirring Hot Plate	VWR® 7"x7"
Dissecting Forceps	8-inch and 4-inch, broad-tipped forceps
Glass Beaker	1,000-ml
Storage Box (Cardboard)	5"x4"x10.5"
Ultra Fine Point Permanent Marker	Sharpie®
Coin Envelopes	4.25"x2.5"
Paper Towels	
II. Making resin block:	
Resin Pump	West System 306-25 Metering
Resin Mixer	Badger Air-Brush 121
Silicone Tray	Lawei 40-well
Epoxy Resin	West Marine Pro, #105-B
Slow Hardener	West Marine Pro, #206-B
III. Sectioning spine:	
Leica MZ 95 or Leica MZ12 Stereo-microscope	Transmitted light source and polarizing filter
Buehler® IsoMet™ Low-Speed Saw	Model 11-1280-160
Steel Flanges	75 mm diameter
Steel Spacer	0.5 mm thickness, 75 mm diameter
Diamond Grinding Wheel	MTI 4"
Allen Wrench	1/8 inch
Weights	Buehler/1180S33, 25 g
Microscope Slide	Premiere 75x25x1 mm
Microscope Slide Storage Box	VWR 28511-012, 100 slides per box
Aluminum Slide Tray	VWR 48467, 20 slides per tray
Glass Eye Dropper and Dropper Bottle	VWR 14216-246, Barnes
Dissecting Forceps	VWR 82027-398, 1 fine point, 1 broad tip
Cordless Precision Engraver	General Tools and Instruments 505
Flo-Texx® Liquid Cover Slip	Lerner Laboratories, 1 quart
Wipers	Kimwipes® Delicate-Task or VWR Light-Duty