# Guide for Processing and Ageing Largemouth Bass Dorsal Fin Spines in Florida

Photo by Duane Raver

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PURPOSES:

1. To introduce the methodology and streamline the process by which Florida Bass and Largemouth Bass dorsal spines are aged.
2. To aid biologists prior to and during a dorsal spine ageing session.
3. To train other scientists so nonlethal ageing may be used at other locations and bodies of water.

MATERIALS LIST:

- A pair of sharp scissors or pocketknife
- Diagonal cutting pliers
- Coin envelopes
- Pencil
- Permanent marker
- Hot plate
- Heating vessel
- Forceps
- Teasing needle
- Fully frosted microscope slides, we use Fisherfinest™ Premium
- Frosted microscope slides, we use Fisherbrand™
- Super glue, we use Loctite® Gel Control™
- Low-speed saw with a weighted arm (South Bay Technologies Model 650 Low-Speed Diamond Wheel Saw), this company is no longer in business, so I would recommend Buehler's® IsoMet™. You can find more in *ordering otolith processing supplies* in FWRI's Ageing Freshwater Fish in Florida guide (hereafter referred to as AFFIF in this document; indexed under Benton, as editor, in References).
- Flanges that support the blades (these usually come with the saw)
- Saw chuck to hold the slide during sectioning (usually comes with the saw)
- Three diamond wafering blades, we use 3" Norton or Buehler®
- Spacers, ours are custom made at 0.5 mm, I would recommend metal rather than plastic
- Coolant for saw, we use Cool 3 by Buehler®
- Mounting medium, we use Thermo Scientific™ Shandon™ Synthetic Mountant or I have used Flo-texx®
- 1-mL plastic dropper or small plastic bottle with squeeze top
- Fume hood or fan
- Dissecting microscope to 45× magnification
- Reflected or transmitted light source
- Beaker or cup to stand dropper upright
- Holding tray for slides (when super glue or mountant is drying)
- Datasheet
- Microscope slide boxes (to store the samples long term)
- Compound microscope 25–100× *optional

- Camera and computer screen *optional, I have used a 10MP USB 2.0 MU1000 AmScope™ microscope camera with AmScope™ 3.7 software and a QImaging MicroPublisher 3.3 microscope camera with QCapture Pro 7 software
- Dressing stick to clean and sharpen blades *necessary for new blades and can renew the surface of old blades for faster cutting
- Quart size plastic bags *optional
- Toothbrush *optional
- Stapler *optional

1. Removing dorsal spines
    a. Raise the dorsal spines. Use a pair of scissors or a pocketknife to cut the skin/webbing connecting dorsal spine III to spines II and IV (Figure 1 left).
    b. Use a pair of cutters to cut as close to the base of dorsal spine III as possible (Figures 1 right, 2, and 3).
        i. This increases the likelihood that all annuli will be visible in the spine sections.
        ii. Annuli can be cut off if too distal of a cut is made (you can read more about this in Murie et al. 2009 and *pages 3–23 and 3–24 in section 3.6.4.2* in AFFIF).

Figure 1. Left: Use scissors to cut the skin anterior and posterior to dorsal spine III. Right: Use cutting pliers to clip dorsal spine III at its base.

Figure 2. Left: dorsal view of the area flush with the back of a Largemouth Bass 35 days after dorsal spines III–V were excised. Right top and bottom: dorsal spines and webbing regrowth one year after clipping for two Largemouth Bass.


Figure 3. Largemouth Bass dorsal spines I–IX, spines are referred to using roman numerals. The yellow lines show where cuts were made to excise the third dorsal spine.

2. Storing dorsal spines
    a. 1) Store the dorsal spine in a coin envelope where it will dry out or 2) place the dorsal spine in a plastic bag to keep it hydrated and store it in the freezer.
    b. Label the storage bag or envelope internally and externally with the necessary information (e.g., fish ID, TL, weight; Barada et al. 2011).
    **c. Working with the spine in a wet or dry condition is personal preference. Ultimately, the dermal tissues surrounding the spine need to be removed.**

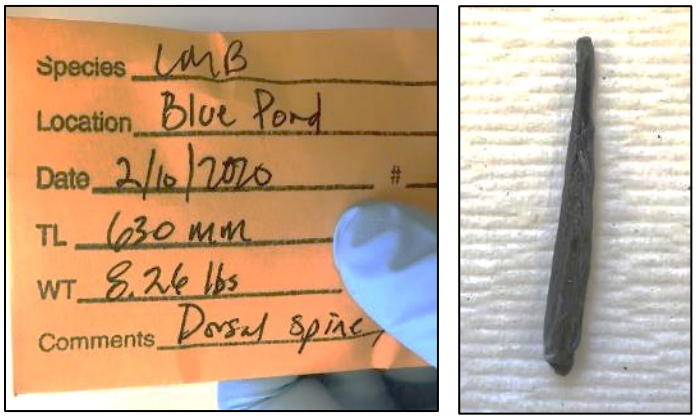

Figure 4. Left: Largemouth Bass dorsal spine stored in a coin envelope. Right: dorsal spine III after excision.

3. Cleaning dorsal spines
    a. If the dorsal spines were frozen, let them thaw before cleaning them. If they were dry, begin cleaning them.
    b. Use a hot plate and water heating vessel. **I use crucibles or a small cup that fits on the top of the hot plate (Figure 5).**
    c. Fill the vessel with hot water and place it on the hot plate.
    d. Set the hot plate temperature to medium (~95°C; Murie et al. 2009) so the water is simmering.
    e. Submerge the spine in the simmering water for ~one minute, and then remove it with a pair of forceps.
    f. Use a gloved fingernail and forceps to strip off the dermal tissue surrounding the base of the spine working basal to distal. Scrape the remaining tissue out of the posterior groove (Allman et al. 2016). You can also use a teasing needle or a toothbrush.
        i. The cleaned spine should look opaque.
        ii. **It is ok to leave tissue on the most distal point because you will not be taking sections from the tip (Figure 5).**

Figure 5. Top left to right: Largemouth Bass dorsal spines submerged in a water filled crucible on top of a hot plate, dorsal spines III–V submerged, and removing tissue with forceps. Bottom left to right: a cleaned dorsal spine, and dorsal spines drying with basal ends exposed in coin envelopes.

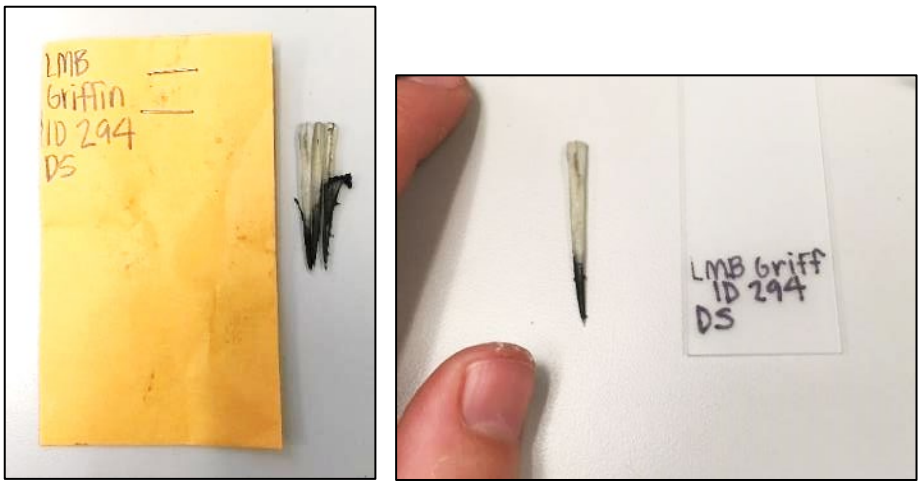


Figure 6. Left: Stapled coin envelope where Largemouth Bass dorsal spines will be set in to dry. Right: dorsal spine III after dermal tissues were cleaned from the outside and posterior groove.

g. Place the clean spine in a coin envelope with the basal end exposed (Figure 5 and 6 left).
    i. **You can staple the top of the envelope and then stick the dorsal spine inside the gap so they will not move (Figure 6 left).**
f. Air dry for ~one day. **You can read more about drying fin structures in *section 3.6.4.2* in AFFIF).**

4. Mounting dorsal spines
    a. Cut or break off the distal most portion of the spine so it fits within the width of a microscope slide (~2.54 cm; Figure 7).

i. Make sure the spine is not hanging off the side of the slide or it will not fit properly in the saw chuck.
ii. **You can use a surgical scissors or other cutting device, but I usually just hold the spine and carefully break off the distal point using my hands. This may not be necessary for spines removed from smaller bass.**

b. Label a fully frosted microscope slide to correspond with the spine.
c. Adhere the whole spine using a thin bead of super glue along the length of its longest surface area, posterior groove (flat) side down, non-tangentially, to the top of the porous side of the slide (Figure 7 bottom).
    i. **Too much glue will result in the sections being harder to remove from the cut spine after sectioning.**
d. Allow the mounted spines to dry on the slide for a minimum of one hour.
e. **There are other methods for preparing spines that include initial embedding. I have used an embedding technique (Lindelien 2018), and there are many options and variations you could explore (e.g., Koch and Quist 2007; *section 3.6.4.2* in AFFIF; Barada et al. 2011 with pectoral spines) By not embedding the spines you save a good deal of time that is normally spent cutting through an epoxied spine using a low-speed saw. It only takes about 20 seconds to section a dorsal spine that is not imbedded.**

Figure 7. Top left to right, Largemouth Bass dorsal spine with broken off distal point, posterior side of dorsal spine III showing the distal point removed to fit on a microscope slide. Bottom: dorsal spines III adhered, posterior groove down, to their respective slides with super glue.

5. Sectioning dorsal spines
    a. Use a low-speed wafering saw (there are many different saw and blade options that are listed with ordering information in *ordering otolith processing supplies* in AFFIF), and diamond encrusted cutting blades (3) used for sectioning otoliths.
    b. Add three blades to the saw separated by two 0.5 mm spacers.
        i. **Saw setup is described in AFFIF *saw arm setup*, if you are unfamiliar.**
    c. If you are using new blades, they need to be dressed (info in AFFIF *dressing blades*).
    d. Place a slide with a mounted spine into the saw chuck (Figure 8).
    e. Line the basal most portion of the spine up with the first blade.
        i. The first blade should be ~1.0 mm from any remaining dermal tissue inside the spine near the cut portion of the base.
        ii. Look for darkness or residual skin. You will want a clear section, so adjust the first blade by moving the micrometer distally as needed (Figure 8). **Read more about this on *page 3-22 section 3.6.4.1.1. and Figure 3.36* in AFFIF. Jearld (1983) describes the importance of sectioning spines just distal of the base (or basal groove via Sneed 1951).**
    f. **Start your saw and then** move the saw arm down with the mounted dorsal spine onto the blades (AFFIF *section 3.4.2.2*).
        i. Allow the saw to cut the spine until you hear it move through bone, glue, and when it hits glass (i.e., the slide), stop the cutting.

Figure 8. Left to right: Largemouth Bass dorsal spine on slide situated in saw chuck and using low speed saw to take sections from a dorsal spine, beginning ~1.0 mm from visible dermal tissue in the basal cut end.

g. Remove the slide from the saw chuck and pat dry any residual liquid with a paper towel.
h. Use a teasing needle to ease out the two sections from the spine. Lift them out with forceps and place them flat side down on a frosted microscope slide (Figure 9).
i. Take as many transverse sections as needed until you garner an ageable one.
    i. You can check the sections with water or immersion oil before permanently mounting them.


Figure 9. Removing two Largemouth Bass dorsal spine sections from the whole spine after cutting has finished. Dorsal spine sections 0.5 mm thick before permanent mounting medium has been applied.

6. Permanently mounting dorsal spine sections
    a. Use a mounting medium and a dropper (AFFIF).

**b. It is highly recommended to use a fume hood depending on the type of mountant. Otherwise, place a fan next to the work area and open a window. Be sure to read the MSDS sheet for your respective mounting medium.**

c. Use the dropper to collect some mountant.
   i. **Collect enough mountant to decrease the amount of times the main container is opened. This will reduce the amount of bubbles trapped in the dropper.**
   ii. **Keep the dropper with the bulb facing upright when moving between sections to avoid this issue. I set the full dropper with the bulb side up inside a cup or beaker where it will stand and not fall over.**
   iii. **Instead of a dropper you can also use a small plastic disposable bottle.**

d. Place the slide under a dissecting microscope and use the dropper to squeeze mountant over the sections.
   i. Spread the mountant completely over the spines using a teasing needle (Figure 10).

**e. This is a good time to double check that there are no bubbles in the mountant that may obscure your view of the spine section. Try to move all the bubbles away from the section as described in AFFIF *processing–Flo-texx.***

f. Be sure that the mountant is level with the edge on all parts of the sections to reduce an artificial edge, which is when the edge of the structure is taller than the amount of mountant covering it.

g. Place the permanently mounted dorsal spine sections into a slide holding tray so they can dry and the mountant can cure for at least 24 hrs.


Figure 10. Largemouth Bass dorsal spine sections that have been permanently mounted to a microscope slide with mountant.

7. Ageing dorsal spine sections
    a. Once the sections are dry, order them, and store them in a microscope slide box with the mounting medium on the slides all facing the same direction.
    b. Use a dissecting microscope (25–45×) and reflected light.
    **c. Transmitted light can be useful, it offers a different view of the sections.**
    **d. If you have access to a compound microscope this can also provide an alternate view especially for older fish. Viewing can be done from 25× to 100× magnification (AFFIF *section 3.6.4.3* considers a compound microscope most optimal for viewing fin structures).**
    e. Place the slide under the microscope and count the number of translucent zones (Chilton and Beamish 1982; Murie and Parkyn 2005). These will appear as light bands with transmitted light and dark with reflected light.
    f. Record the amount of opaque zone (0–4). Opaque zones will appear as dark bands with transmitted light and light bands with reflected light.
8. Interpreting dorsal spine sections
    a. Spines are unsegmented and unbranched (Moyle and Cech 2004). They contain a single part to age with a central blood vessel that runs through a space referred to as the lumen (Figure 11).
    b. Dorsal spines have landmarks that look like lobes and grooves (Murie et al. 2009). Generally, two lobes are present: one elongate and one compacted (Borkholder and Edwards 2001). Two grooves exist: one on the anterior edge and one on the posterior edge.
        i. The elongate lobe has been more prone to display checks (Jearld 1983 describes checks and split bands) and double-or multi-bands possibly because of more surface area for formation.
        ii. The compacted lobe is smaller and can be easier to interpret.
        iii. The anterior groove is located at the front of the spine when it is raised on the fish.
        iv. The posterior groove is located at the back of the spine where it connects to the adjacent spine on the dorsal fin (Figure 11).
    c. Dorsal spines form translucent (absorptive zone; Casselman 1974) and opaque (reflective) zones, just like otoliths (AFFIF *otolith structure and function*).
    d. In dorsal spines the translucent zones are counted, and they indicate slow growth just as opaque zones do in otoliths.
    e. Opaque zones represent fast growth which are assigned margin codes (0–4; AFFIF *section 4.2.1*).

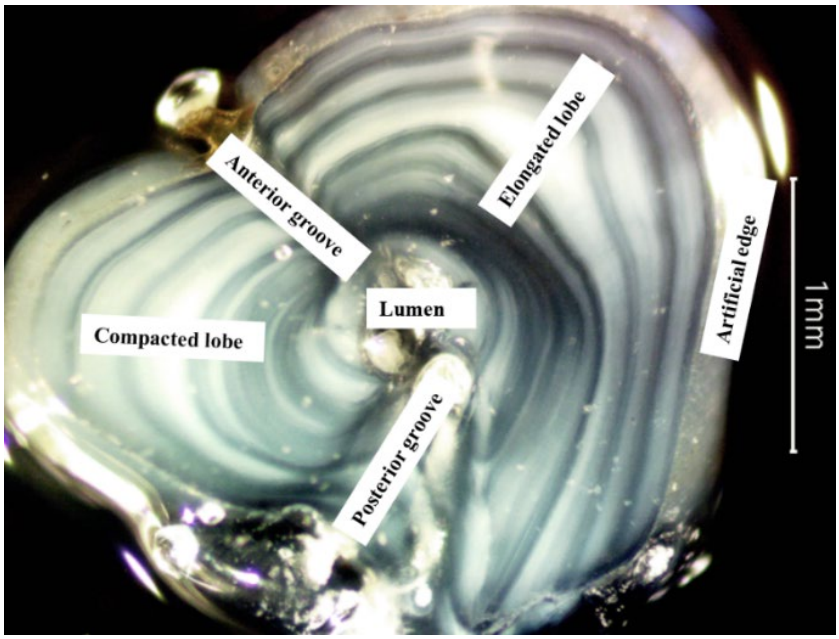


Figure 11. Dorsal spine section from a seven-year-old Largemouth Bass displaying an artificial edge, the two lobes and grooves, and central lumen. Viewed with reflected light.

9. Nuances
    a. Double- and multi-banding
        i. Multiple translucent zones can fuse in certain areas to form one whole year of growth that should be aged as such ("doublets" in Allman et al. 2016).
            - Look for joined translucent zones in the grooves (Figure 12 left; Hedgepeth and Jolley 1983), and if they are not present there but present on the lobes, we would generally count them as one year of growth.

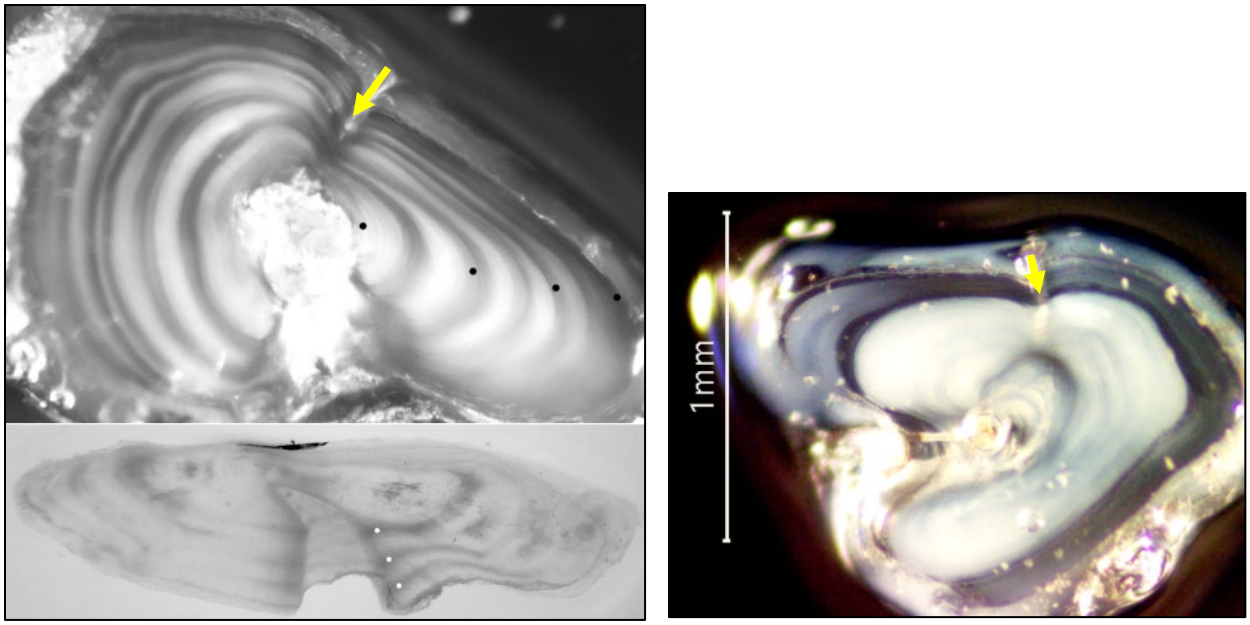


Figure 12. Left: top photo shows a dorsal spine section with double-banding pattern, black points represent enumerated translucent zones (annuli), yellow arrow represents area where translucent zones run together as one annulus. Bottom photo shows the otolith section from the same four-year-old Largemouth Bass without double-banding pattern, white points represent counted opaque zones. Right: double translucent zone (annulus) formed in a dorsal spine from a two-year-old Largemouth Bass. All sections were viewed with reflected light.

b. Core expansion and annulus occlusion
    i. The lumen or central space is filled with a blood vessel when the fish is living. This blood vessel can expand over the first few annuli as resorption occurs (Figure 13; Casselman 1983) and the bony tissues are broken down/eroded and mineralized as the fish grows. This can cause the fish to be underaged because annuli are occluded from view when the blood vessel has nowhere to go; therefore, it moves into the space where annuli were.
    ii. Refer to *page 3-21* and *section 3.6.4* in AFFIF for more information regarding core resorption.

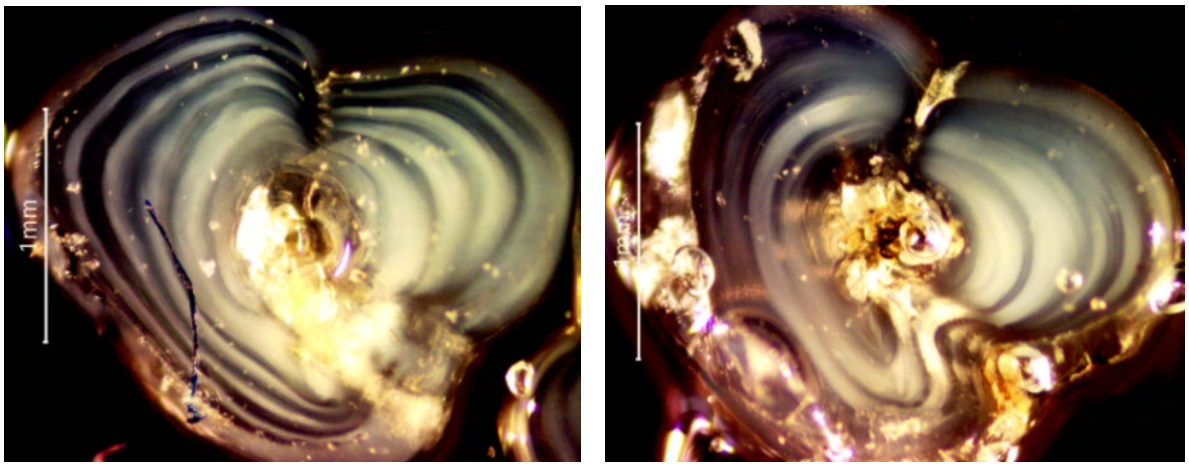


Figure 13. Two Largemouth Bass dorsal spine sections displaying core resorption and erosion. Viewed with reflected light.

c. Edge crowding of annuli
    i. The edge of the structure is where translucent and opaque zones sometimes stack on top of one another or compact in older fish (Chilton and Beamish 1982; Casselman 1983; Murie et al. 2009). It can be difficult to count and separate marginal zones causing under ageing of old fish. Using a compound microscope or projecting images on a screen can help readers identify and separate annuli (Figure 14; *section 3.6.4.3* AFFIF).

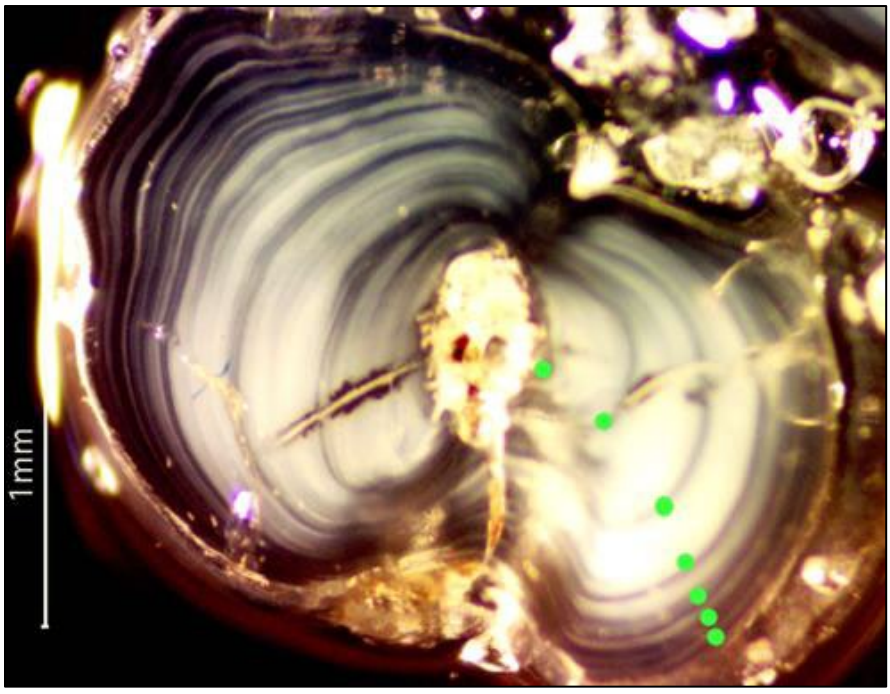


Figure 14. Edge of a Largemouth Bass dorsal spine on lower part of compacted lobe next to posterior groove contains bands that run close together, green points represent counted translucent zones. Viewed with reflected light.

d. Faded annuli
    i. The first few annuli were visibly faint in some older specimens. This could be due to the translucent zone being reabsorbed as the fish ages. Or the fish grew so quickly annuli were laid fast and less distinctly.

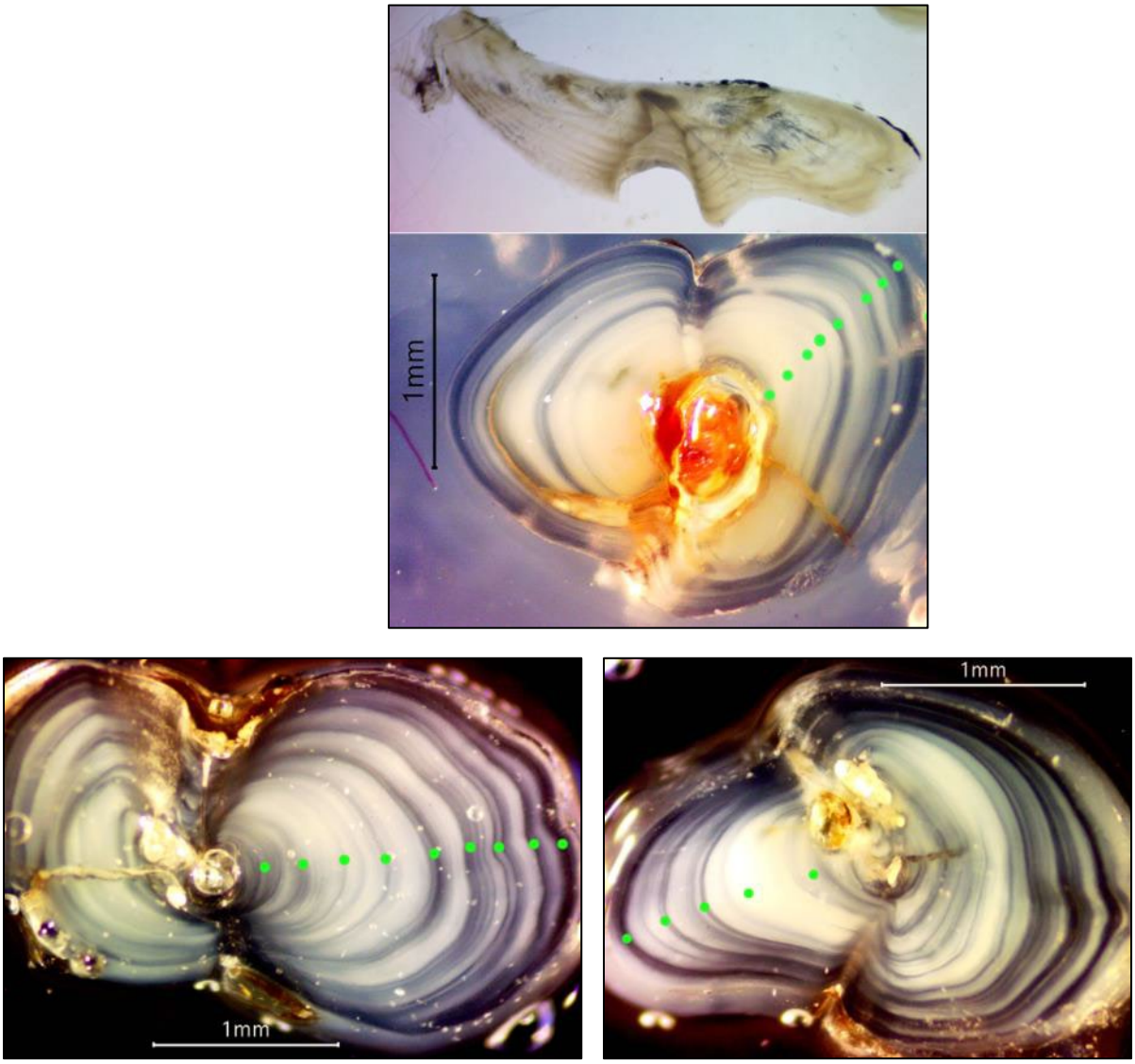


Figure 15. Faded translucent zones in Largemouth Bass dorsal spines, green points represent counted translucent zones, viewed with reflected light. Translucent zones 3 and 4 are faded in the left photo. Translucent zones 1 and 2 are faded in the right photo.

e. Checks or false annuli
    i. When a fish undergoes a change, it can cause growth to slow down; thus, a band may form that is not annular (Jearld 1983).
        - May result from a variety of intrinsic or external factors including spawning, changes in prey, and changes in water temperature or

water levels (Evans and Hoenig 1998; Donabauer 2010; Snow et al. 2018).

ii. Some checks were probably also visible if the dorsal spine was sectioned tangentially (Chilton and Beamish 1982) or not cleaned properly before transverse sectioning.

iii. Read about checks in Chilton and Beamish 1982; Jearld 1983, and *section 3.6.4* AFFIF.

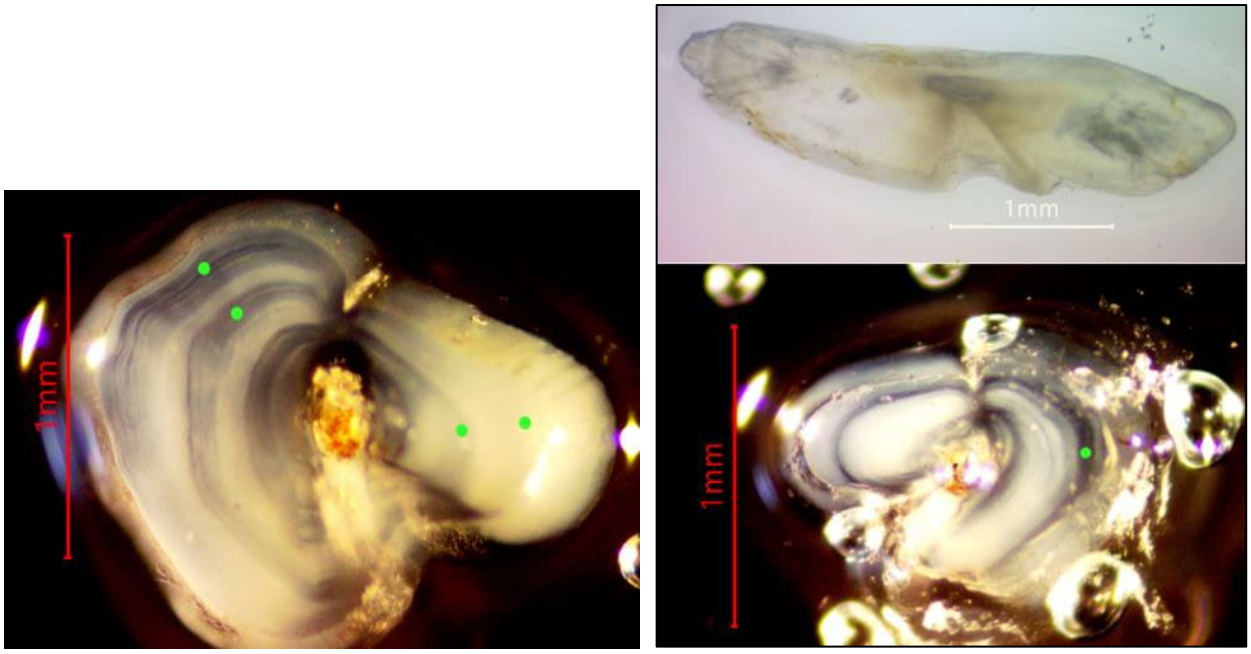


Figure 16. Left: checks within the core and first year of growth in a dorsal spine section from an age-2 Largemouth Bass, green points represent counted translucent zones, viewed with reflected light. Right: check within the first year of growth of a one-year old bass.

10. Timing of growth zone formation

a. Based on a marginal increment analysis of age 3, 4, and 5 Largemouth Bass dorsal spine sections, translucent zones begin forming in the fall months (October, November, and part of December).

i. This could vary across waterbodies based on latitude (Jearld 1983).

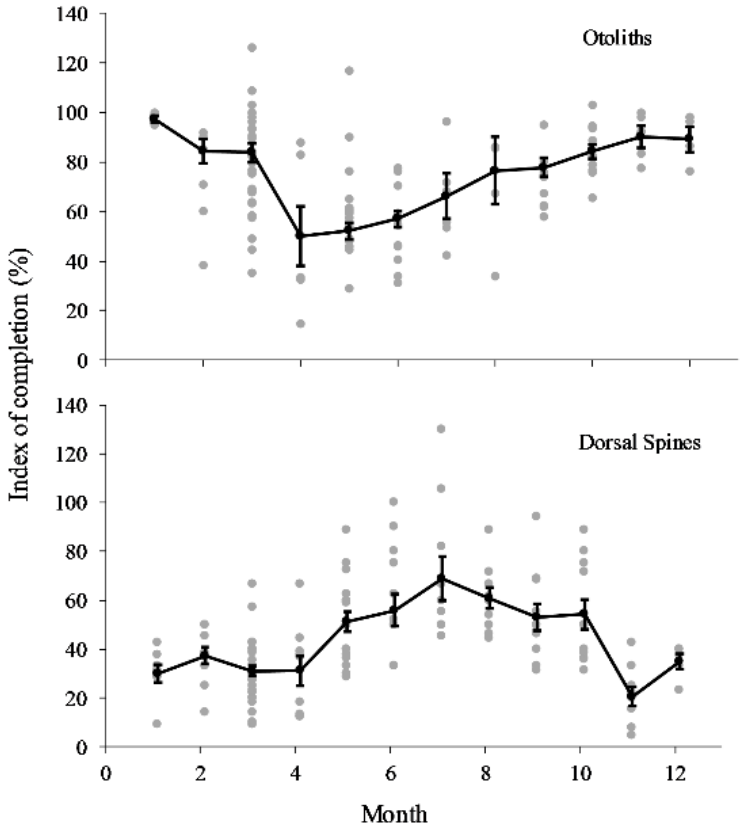


Figure 17. Top: index of completion for age-4 Largemouth Bass sagittal otolith sections ($n$ = 83) across 12 months spanning February 2017–January 2018. ±1 standard errors are vertical increments around each monthly mean represented as black points and line. Grey points represent the index of completion for individual fish in each month. Bottom: index of completion for ages-3, -4, and -5 Largemouth Bass dorsal spine sections ($n$ = 151).

Figure 18. Sections of Largemouth Bass dorsal spines displaying an opaque zone on the edge or just beginning translucent zone (annulus) formation, viewed with reflected light.

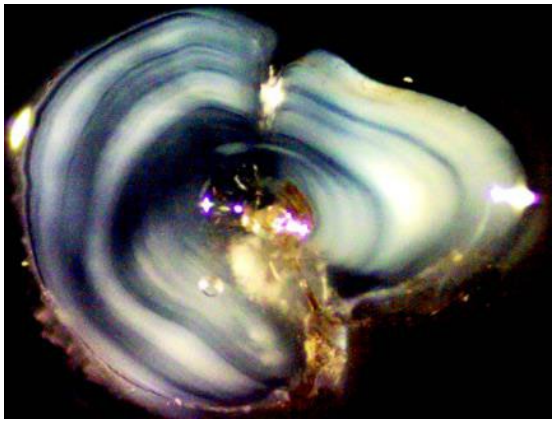

Figure 19. Largemouth Bass dorsal spine section displaying translucent zone (annulus) formation on the edge, viewed with reflected light.

b. Some dorsal spine sections collected in the fall may have already begun forming a translucent zone on the margin.
c. Some spines sampled in the fall may still have a wide opaque zone on the margin. Thus, we recommend using margin codes as described in AFFIF *section 4.2.1*.
    i. Number of translucent zones may not directly transfer to age. Researchers who wish to use integer ages should carefully consider whether to round ages up or down.
d. If we collect spines in the spring, we expect the marginal translucent zone to be fully formed, and fish age would be equal to the number of translucent zones visible.
e. Translucent zone formation begins 6 to 7 months prior to initiation of the opaque zone in otoliths, which forms in the spring (Figure 20).
f. Chemical marking with OTC would validate translucent zone formation on an annual basis in Largemouth bass dorsal spines (Allman et al. 2016).

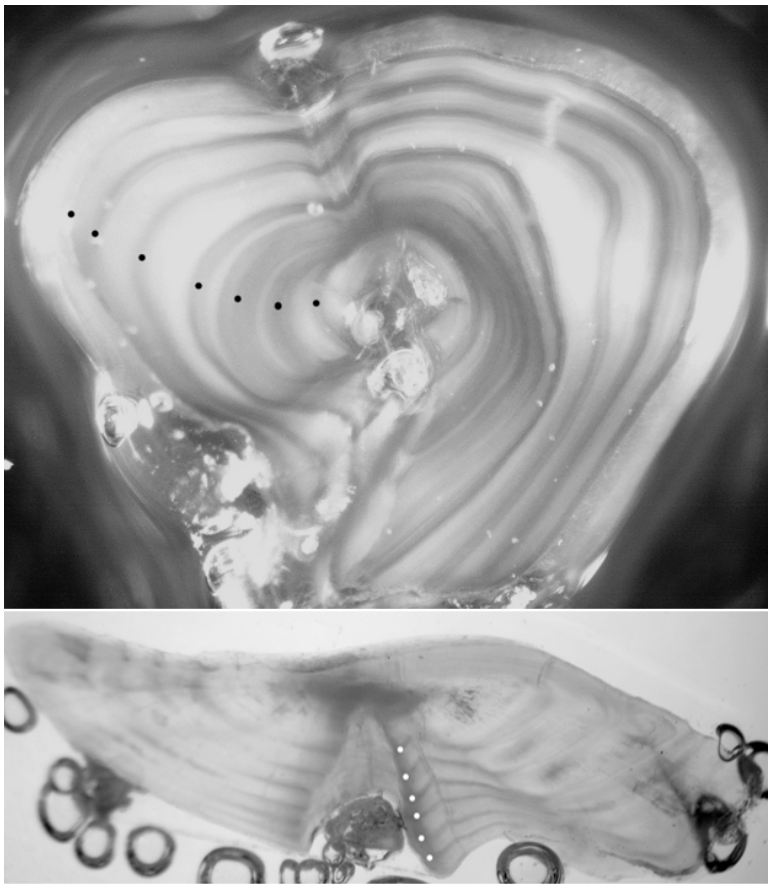

Figure 20. Top) Largemouth Bass dorsal spine section viewed with a dissecting microscope and reflected fiber optic light source at 40×. Black points indicate enumerated translucent zones. Bottom) Sagittal otolith section viewed with a dissecting microscope and a transmitted LED bottom light source at 30×. White points are enumerated opaque zones. Based on marginal-increment analyses, the dorsal spine section will display its slow growth/translucent zone prior (~6 to 7 months) to the sagittal otolith (opaque zone), hence the difference in counted points for the same fish.

11. Reference sets
    a. Our reference sets were subsamples of paired bass dorsal spines and otoliths that were photographed and marked to aid readers during an ageing session.
        i. Each reference set consisted of a representative distribution of lengths and ages that were specific to each of the seven waterbodies assessed in Florida.
        ii. **You can read more about reference sets in Campana (2001), Dembkowski et al. (2019), and building a reference collection in *section 4.4.4* in AFFIF.**
        iii. **Read about further reasoning and the commonality of using references sets for training in Allman et al. (2016).**

b. We used reference sets specifically to train ourselves how to identify translucent and opaque zones in dorsal spines from different waterbodies in Florida so that all readers were following similar ageing criteria.
c. We also referenced the subsamples during our ageing sessions to try and increase our agreement with otolith ages.
    i. To build a reference set
        - FWC opportunistically collected bass during typical age sampling events.
            - We chose 15 fish from a sample of 60 that included a uniform distribution of sizes, chosen by length using a stratified random process.
                - This was a minimalist approach; whereas, you could collect more fish (e.g., two to five fish per cm group).
            - **Be sure to include enough short/young fish because these fish will help guide the correct identification and interpretation of the first annulus in dorsal spines.**
        - Process both the dorsal spines and otoliths from your collected bass to create the subsample for referencing a waterbody.
            - **Be sure you give both structures an ID and they remain matched and linked to the same fish.**
        - Age the dorsal spines and otoliths and keep a document with the correct information.
        - Archive the paired samples for future reference and training purposes for nonlethal ageing where it can be specific to waterbodies commonly sampled by your office.
        - You can always add more samples to your reference set to include all necessary sizes of fish.
        - It can be transferred to other offices for training and viewing.
d. Ideally, once you have trained with a specific waterbody reference set, you may only have to sacrifice bass for otoliths from certain lengths and take dorsal spines to age fish longer than a length cutoff.
    i. These cutoffs will depend on the waterbody and length at age.
e. In order to optimize nonlethal ageing, you will need to learn proper ageing criteria of dorsal spines in your waterbody. This requires you to sacrifice fish for otolith and spine comparison to build a reference set.
f. **It is not mandatory to use an imaging system; you could just process the spines and have them available to look at under the microscope while ageing instead of in photograph form.**
g. **The reference set should include average examples of both otoliths and dorsal spines to train from.**
    i. **We would not want to solely include "good" or clear sections in our set because we want some unclear samples that we will encounter in**

**our typical age sample (you will be collecting a stratified random length subsample of the fish encountered).**

h. First annulus establishment
    i. Frequently documented, a difficult issue can be identifying the first annulus (Chilton and Beamish 1982; Isermann et al. 2003; Allman et al. 2016; Dembkowski et al. 2017; Dembkowski et al. 2019).
        - This can be circumvented by analyzing some shorter fish to help mold your ageing criteria of older fish.
    ii. When reviewing your reference set, try to begin by ageing the shorter/younger fish and familiarizing yourself with the first years of growth (Allman et al. 2016), this way you can practice recognizing where the first annulus is for a waterbody and then work up in ages from there.

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