Technical Report

Massachusetts Division of Marine Fisheries
Technical Report TR-58

Massachusetts Division of Marine Fisheries Age and Growth Laboratory: Fish Aging Protocols

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Department of Fish and Game
Massachusetts Division of Marine Fisheries

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Massachusetts Division of Marine Fisheries Technical Report Series

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Abstract: The Massachusetts Division of Marine Fisheries Age and Growth Lab was established and constructed in 2009. The main goal of the lab is to provide age data of recreationally important fish species for use in stock assessments and fisheries management. Age data are important as they are needed to calculate growth rates, determine age of recruitment, determine age of maturity, track cohorts of fish, and calculate mortality estimates.

Conducting all of the Division's fish ageing in one location with consistent staff increases efficiency and precision. All structures are aged by two readers with no knowledge of fish length or sex. Disagreements between the two readers are resolved by both readers re-examining the structure together and coming to an agreement. Quality control procedures include the use of reference collections, bias plots, and precision checks. The coefficient of variation (CV) is calculated between readers to assess precision. A CV of under 5% is targeted and achieved regularly. The lab routinely processes age structures from alewife (*Alosa pseudoharengus*), blueback herring (*Alosa aestivalis*), American shad (*Alosa sapidissima*), rainbow smelt (*Osmerus mordax*), striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*), tautog (*Tautoga onitis*), winter flounder (*Pseudopleuronectes americanus*), and black sea bass (*Centropristis striata*). Significant work has gone into investigating the multiple age structures available from each species as well as the techniques for processing those structures. This document gives an overview of the ageing methods for each species the lab works with.

River Herring

Alewife (*Alosa psuedoharengus*), Blueback (*Alosa aestivalis*)

River herring are anadromous fish, which are most frequently sampled in rivers during their spawning migration. Most fish sampled are three to six years old, although two-year-old blueback herring are not uncommon.

Scales and otoliths are collected. Otoliths yield superior ageing precision over scales, and are preferred when available. Scales can be partially resorbed when fish enter fresh water to spawn. The marks left by that resorbtion can be used to identify spawning checks (Cating 1953). Spawning check data are valuable to management, therefore, our lab processes scales as well as otoliths.

Sample Collection

- Each fish is given a unique sample ID (river, year, and fish number).
- Weight, fork length, total length, sex, species, capture date, and sample ID number are recorded on envelopes and data sheet.
- Species is determined by the color of the peritoneum and the relative size of the eye.
- Otolith extraction:
  - o Using a scalpel or knife, the top part of the head is excised to expose the brain cavity.
  - o The cut begins at the back of the skull, slicing forward through a point just above the eyes (Figure 1a).
  - o Using forceps, the brain matter is removed with a forward scooping motion.
  - o The otic membranes, one on each side, are extracted with fine tipped forceps (Figure 1b–c).
  - o The otoliths are usually found within the otic membrane, but occasionally remain in the bony case where the membrane was removed. A stream of water from a squirt bottle can be used to dislodge the otoliths before removal with fine forceps.
  - o Otoliths are rinsed with water and stored dry in labeled microcentrifuge tubes.
  - o For a video of the procedure see: http://youtu.be/wkGeh-q_Jgo?list=UUlqCy8wTkXK_CbKISYkUHxA
- Scale collection:
  - o Scales are collected ventral of the dorsal fin (Figure 2).
  - o The collection area is scraped with a scalpel or knife to remove slime, other fish's scales, and any debris.
  - o 20–30 scales are collected with a clean knife or scalpel and placed into a corresponding envelope.

Otolith Preparation and Ageing

- Preparation
  - o Water is used to clean the otolith if needed.
- Age Interpretation
  - o Otoliths are immersed sulcus side down in mineral oil, on a black background, and viewed under a stereomicroscope with reflected light.
  - o Annuli are defined as continuous hyaline (dark) bands with no breaks (Figures 3-5).
  - o Annuli are counted from the middle outward along the pararostrum or antirostrum.
  - o False annuli typically are not continuous, appear outside of expected areas, lack a defined edge, or connect with true annuli (Figures 5 and 6).

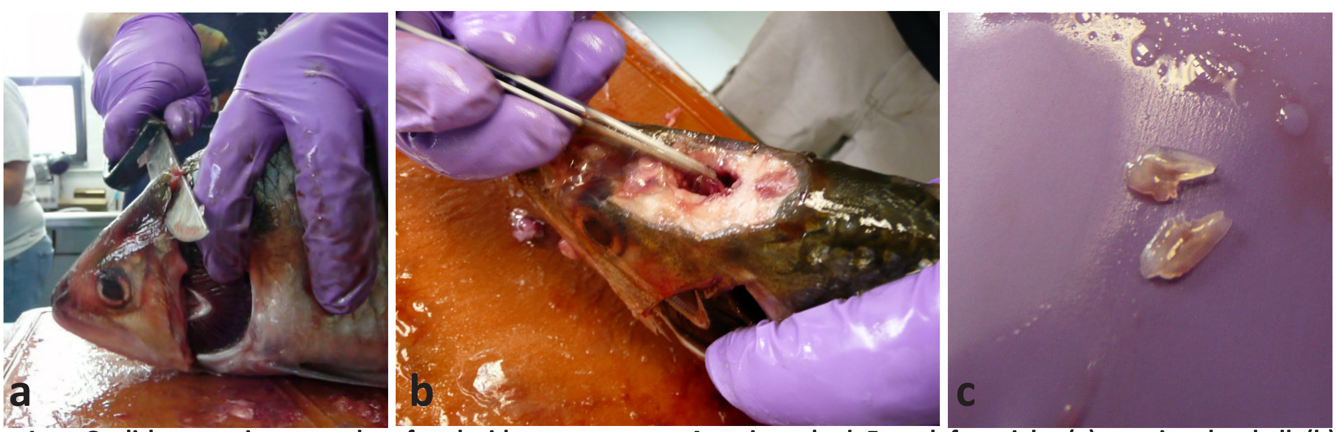


Figure 1a-c. Otolith extraction procedure for alosids, as seen on an American shad. From left to right, (a) opening the skull, (b) removing brain matter, and (c) extracted otoliths.

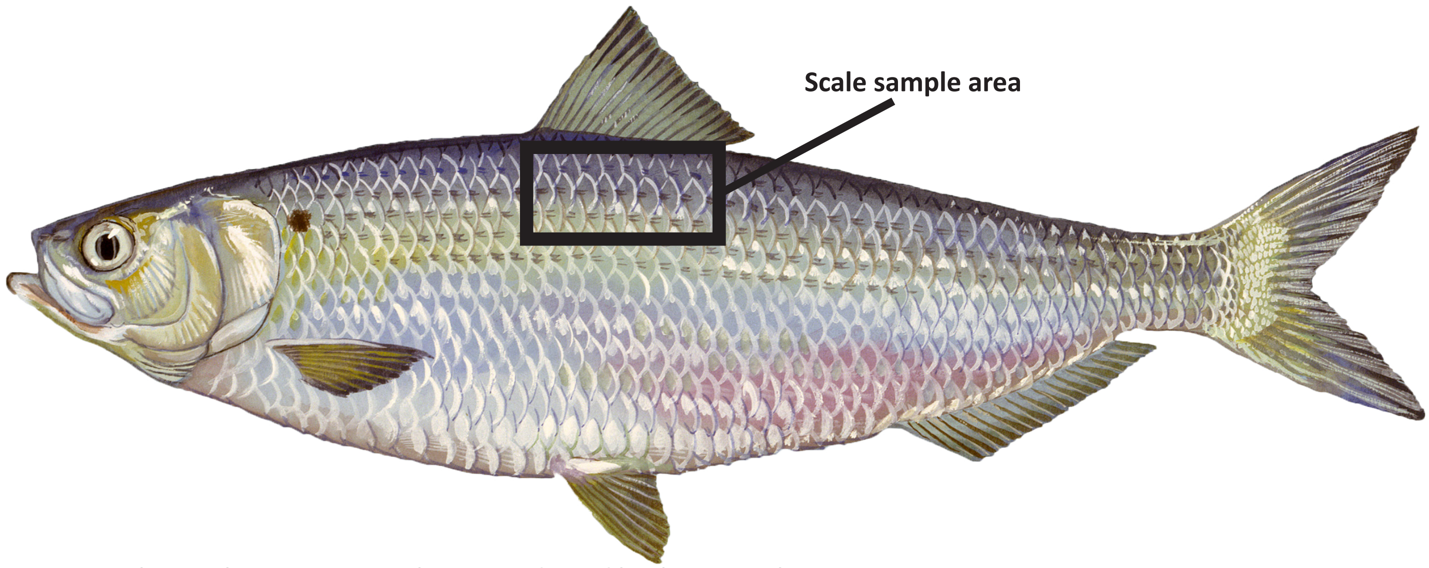


Figure 2. Scale sample area on a river herring, indicated by the rectangle.

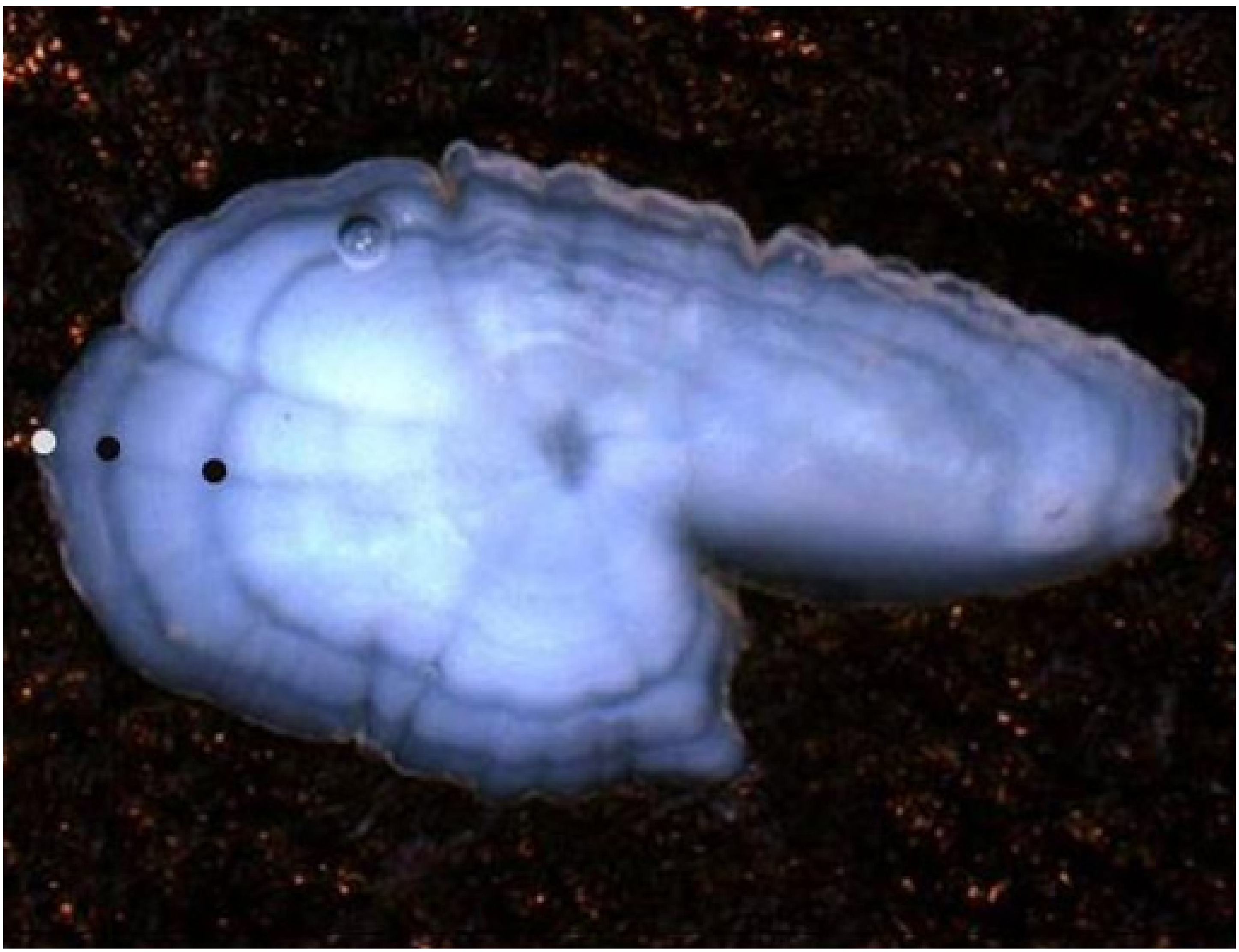


Figure 3. An otolith from a 3-year-old blueback herring. Black dots mark the first and second annuli, and the gray dot marks the edge. The edge is counted in this case, because the fish was caught in the spring.

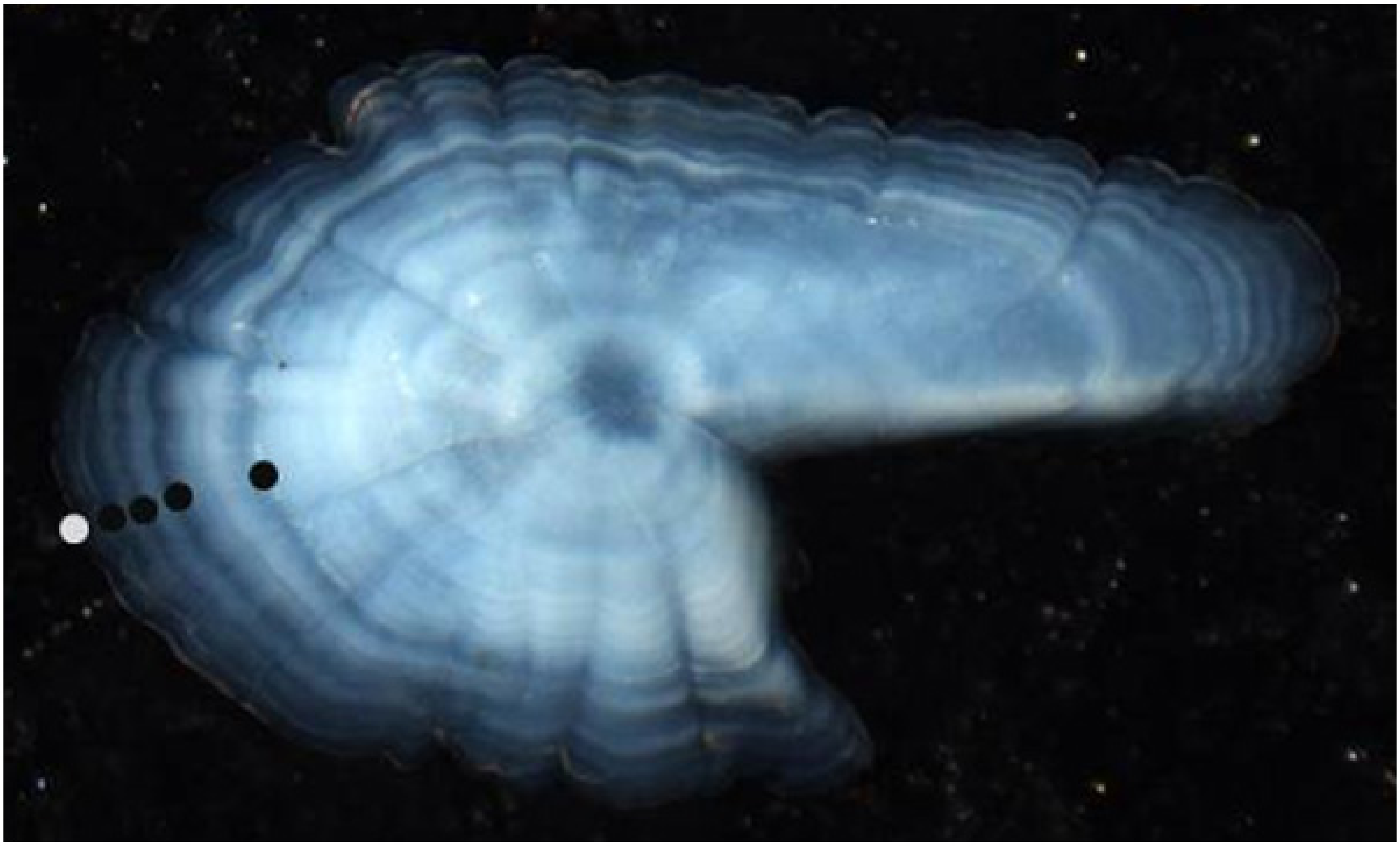
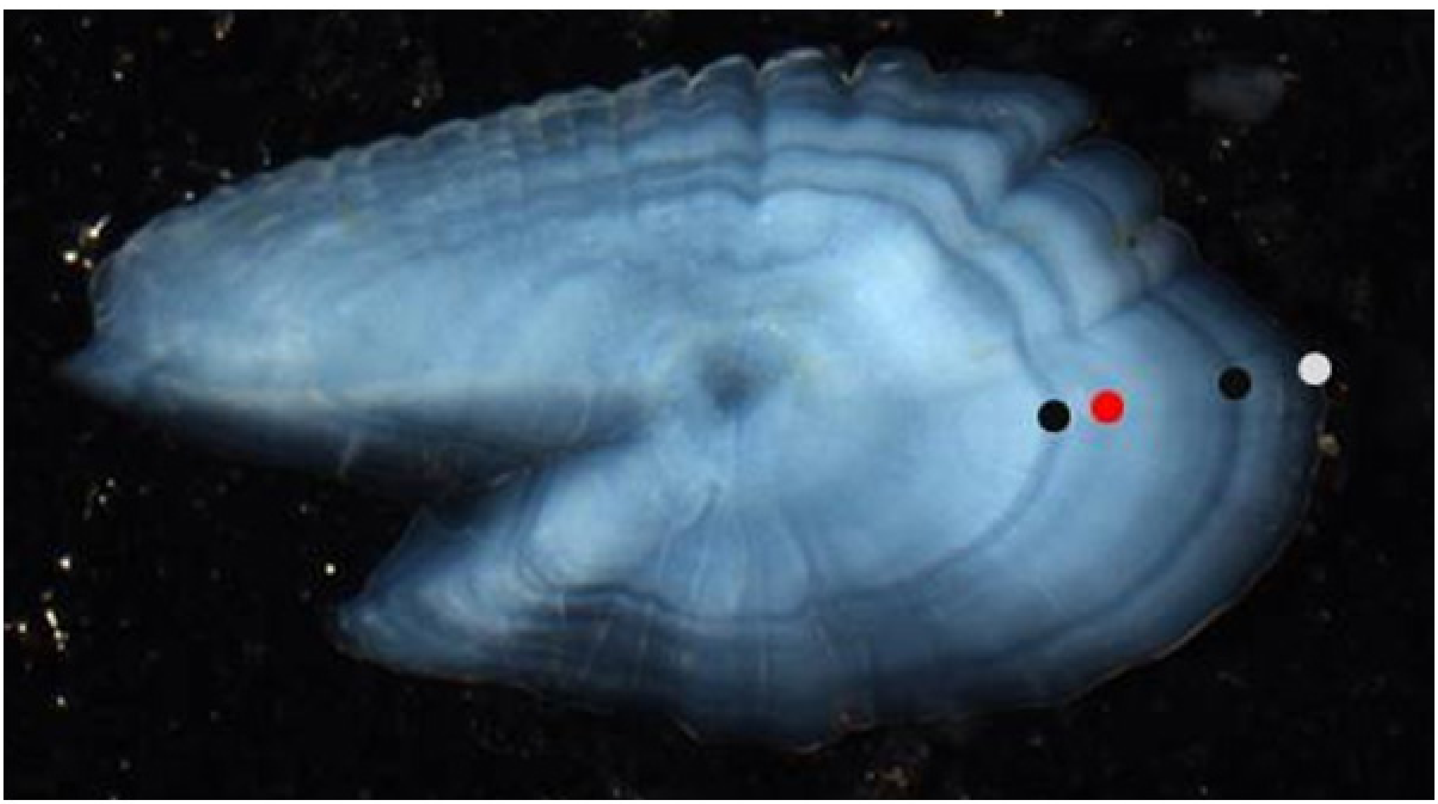


Figure 4. An otolith from a 5-year-old alewife. Annuli 1-4 are marked with black dots and the edge is marked with a gray dot. This fish was caught in the spring, so we count the edge.



Fiugre 5. An otolith from a 3-year-old blueback herring. The red dot marks a false annulus; note how it is not continuously dark. The growth between the first annulus and the false annulus is not as much as expected for this to be a second annulus. The black dots mark annuli 1 and 2 and the gray dot marks the edge. We count the edge because this fish was caught in the spring.

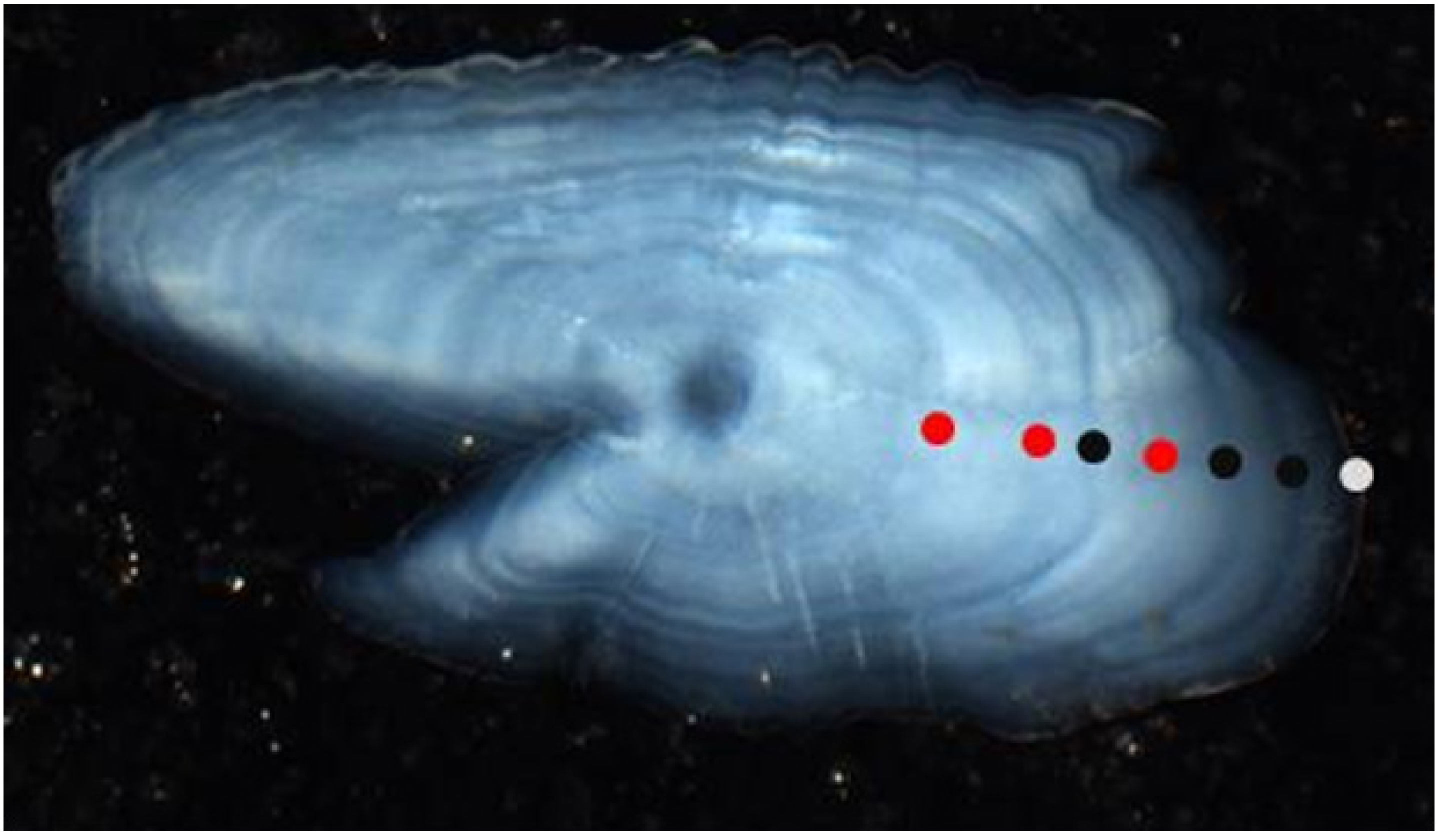


Figure 6. An otolith of a 4-year-old blueback herring. The red dots mark false annuli, the black dots mark the first, second, and third annuli, and the gray dot marks the edge. We count the edge as an annulus because this fish was caught in the spring.

Scale Preparation and Ageing

- Preparation
  - o Six readable scales per fish are selected, regenerated scales are avoided.
  - o A 5% pancreatin solution is prepared and the scales are cleaned in a sonic cleaner following the method outlined by Whaley (1991).
  - o The pancreatin is drained from scales using a small mesh sieve. (The pancreatin can be used several times).
  - o The scales are placed in a dish of clean water, then wiped and dried with paper towels as needed.
  - o The dry scales are placed between two glass slides labeled with the appropriate ID number.
- Age Interpretation
  - o Scales are viewed using transmitted light with image processing software and a camera on a macro mirror stand.
  - o The mirror and lighting are adjusted so the annuli can be viewed crossing over the baseline.
  - o Annuli appear as continuous strong bands that cross the transverse grooves and continue past the baseline.
  - o The first dark band is usually the freshwater zone (Figures 7 and 8).
  - o The first annulus is frequently weak and doesn't always follow the annulus criteria.
  - o The edge is counted as the last annulus if captured in spring (Figures 7 and 8).
  - o False annuli will not cross over the baseline and cannot be followed throughout the scale (Figure 9).
  - o Typically the second annulus is the most well defined annulus (Figures 10 and 11).
  - o On older fish, annuli can become crowded together at the edge of the scale, but will separate beneath the baseline. These should be counted as separate annuli (Figure 12).
  - o Scale resorbtion during spawning can remove parts of previous annuli. These annuli will still separate beneath the baseline and should be counted separately (Cating 1953) (Figure 12).
  - o Spawning marks are identified as annuli that appear fuzzy and jagged above the baseline or appear that they've been resorbed over another annulus above the baseline (Figure 12).

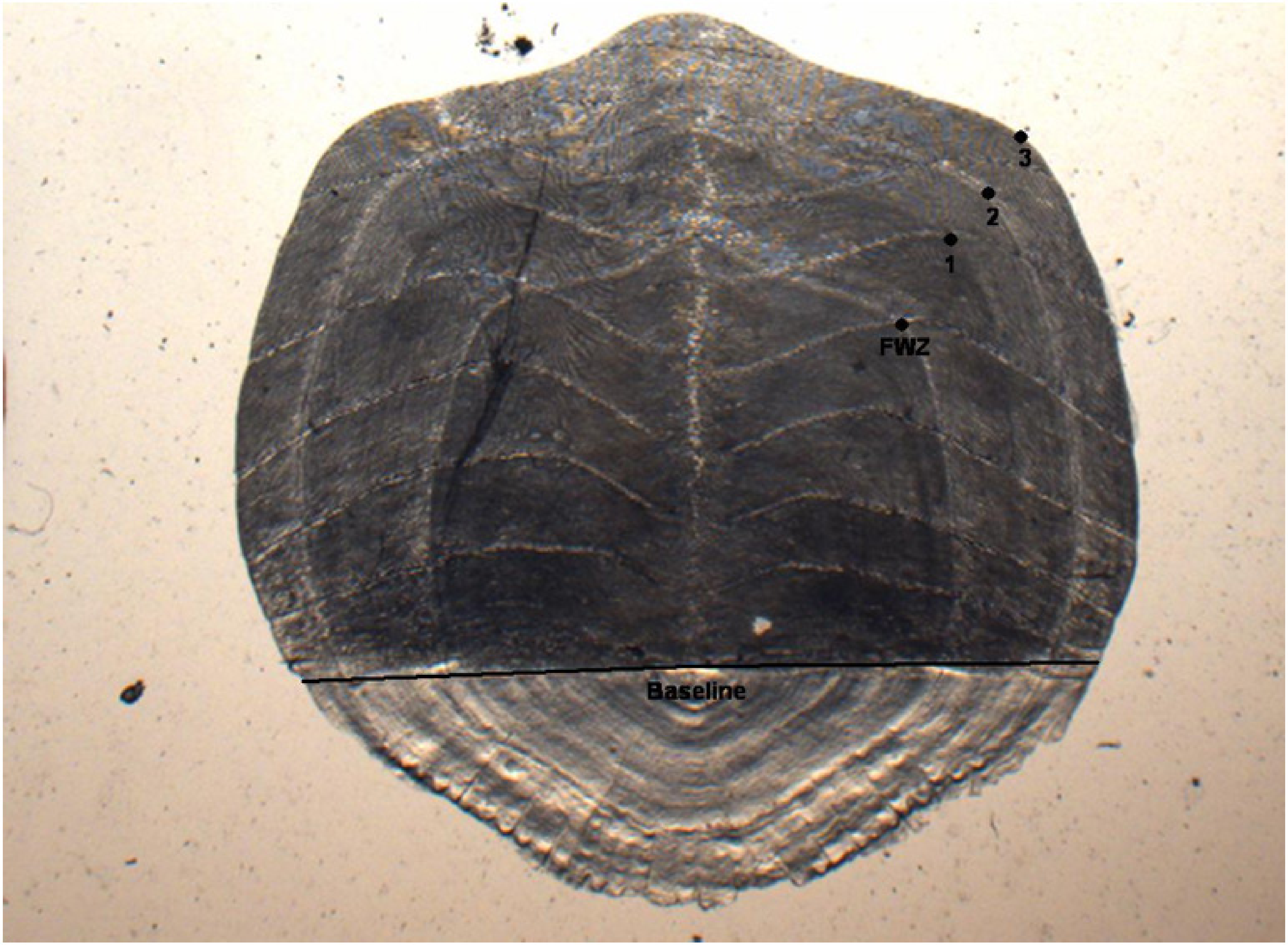


Figure 7. This 3-year-old alewife has the baseline, fresh water zone (FWZ), and annuli marked. Note the straight baseline and large FWZ typical of alewives.

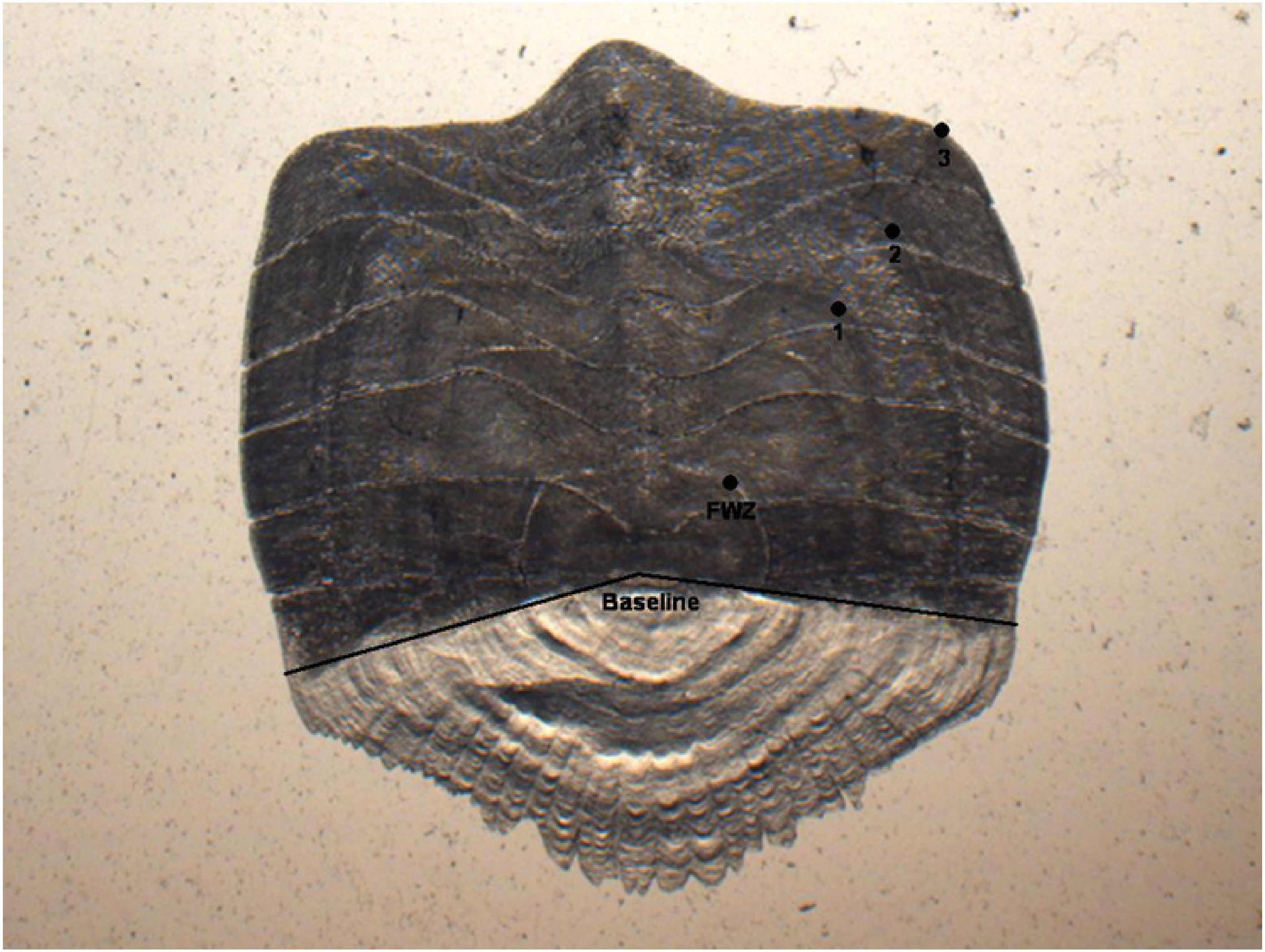


Figure 8. The baseline, fresh water zone (FWZ), and annuli are all marked on this blueback herring scale. Note the small FWZ and angled baseline typical of the species.

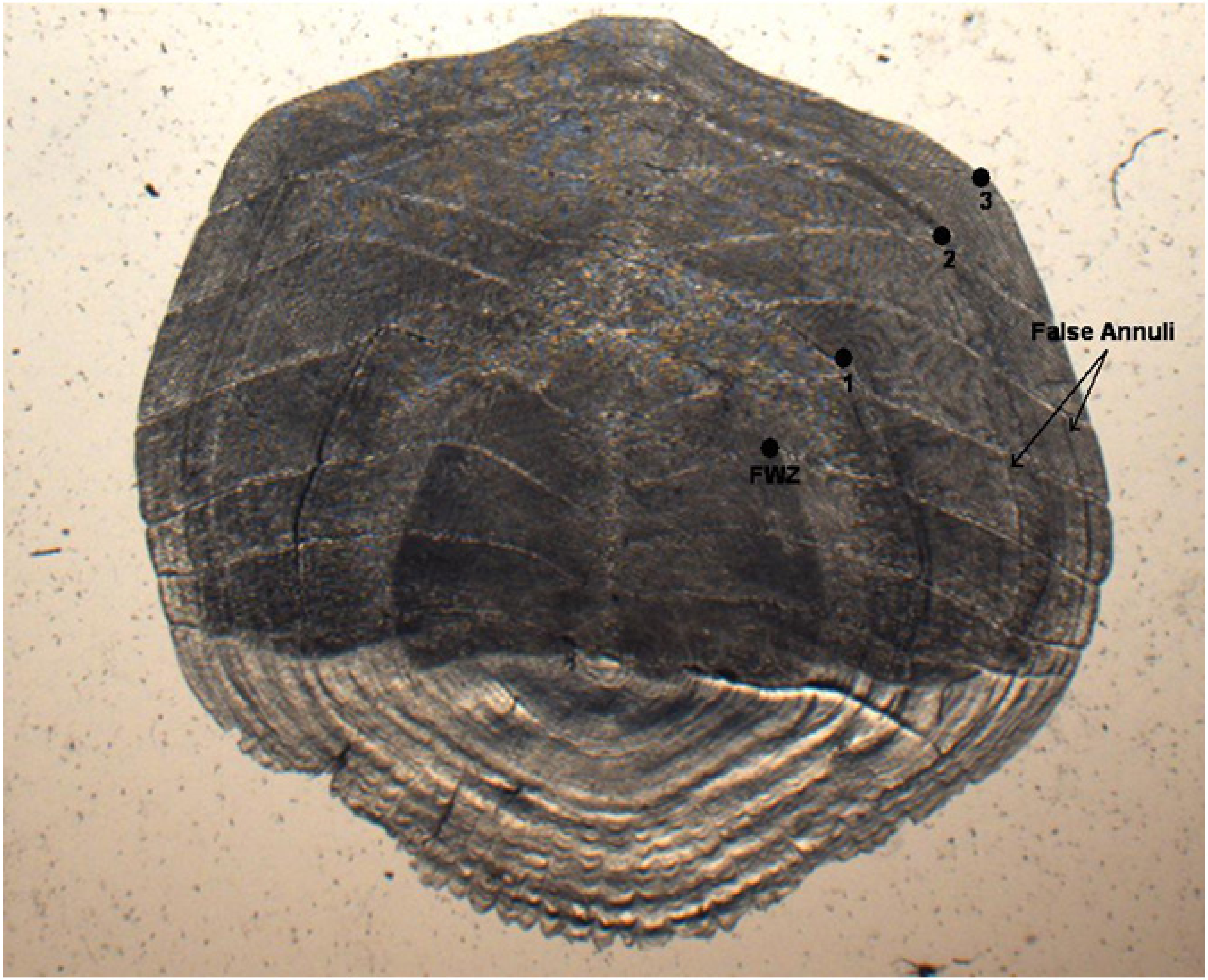


Figure 9. This 3-year-old alewife has two false annuli, one on either side of annulus 2.

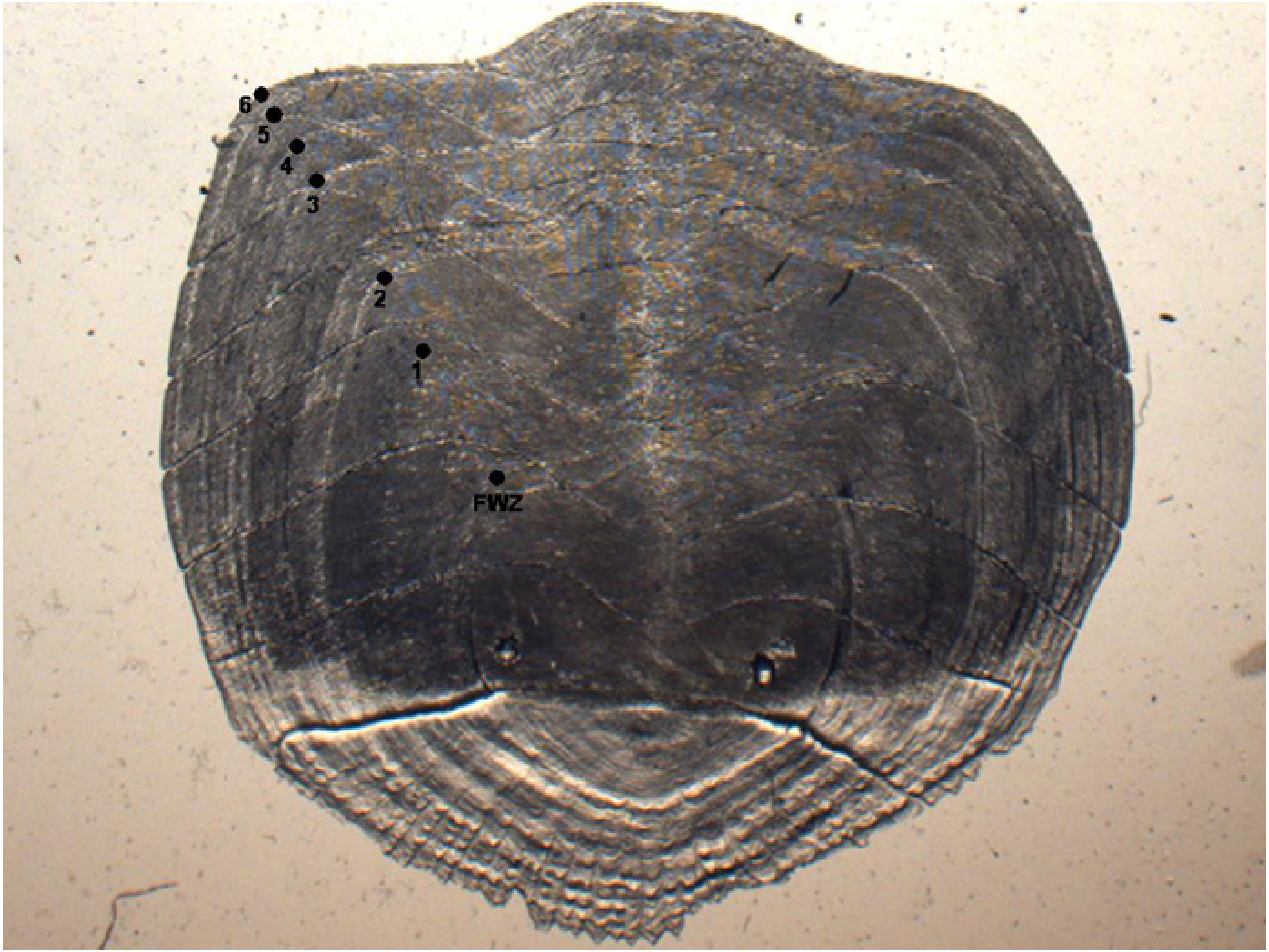


Figure 10. A 6-year-old alewife. Note how weak the first annulus appears compared to the second.

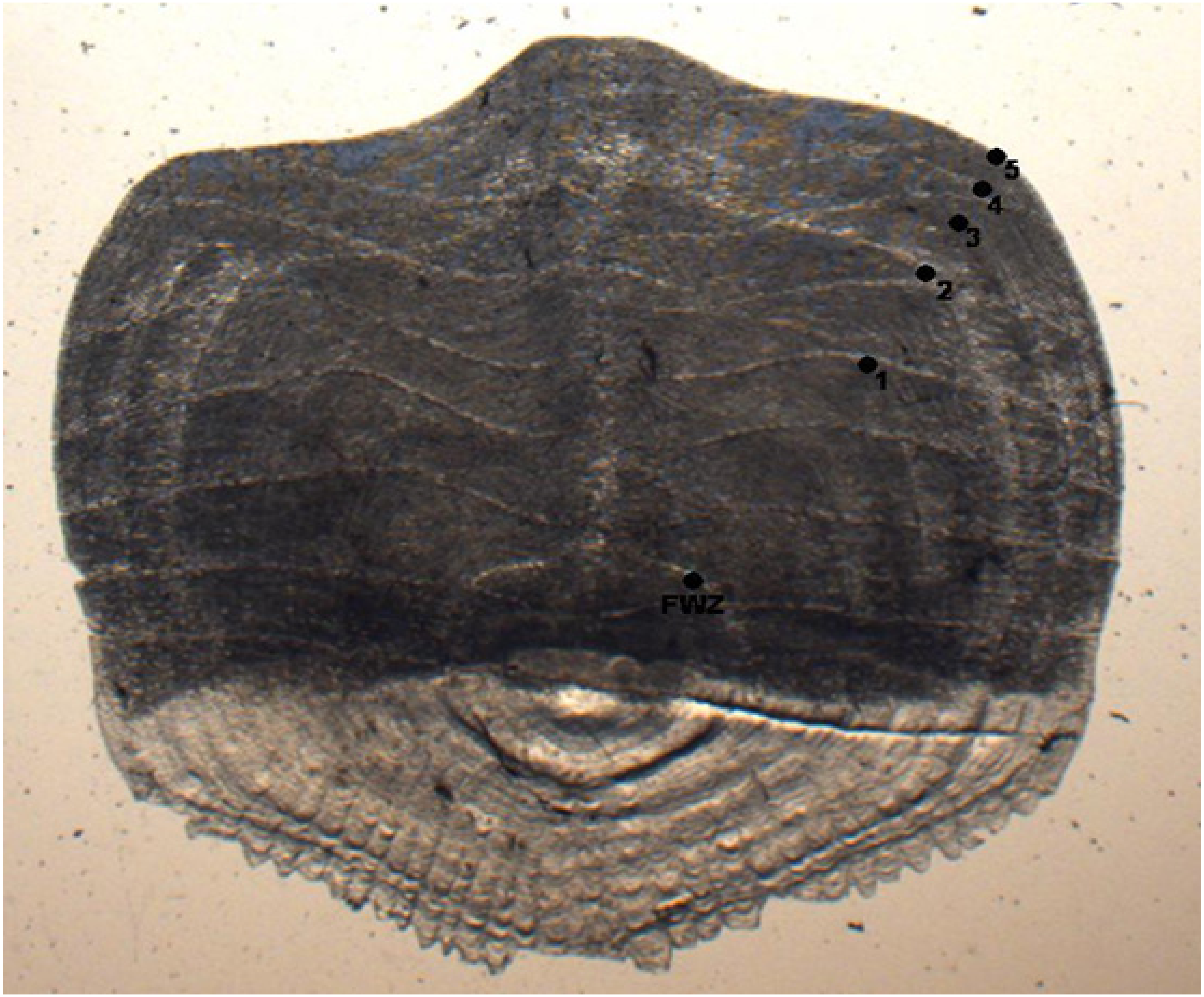


Figure 11. This 5-year-old blueback herring has the typical strong second annulus.

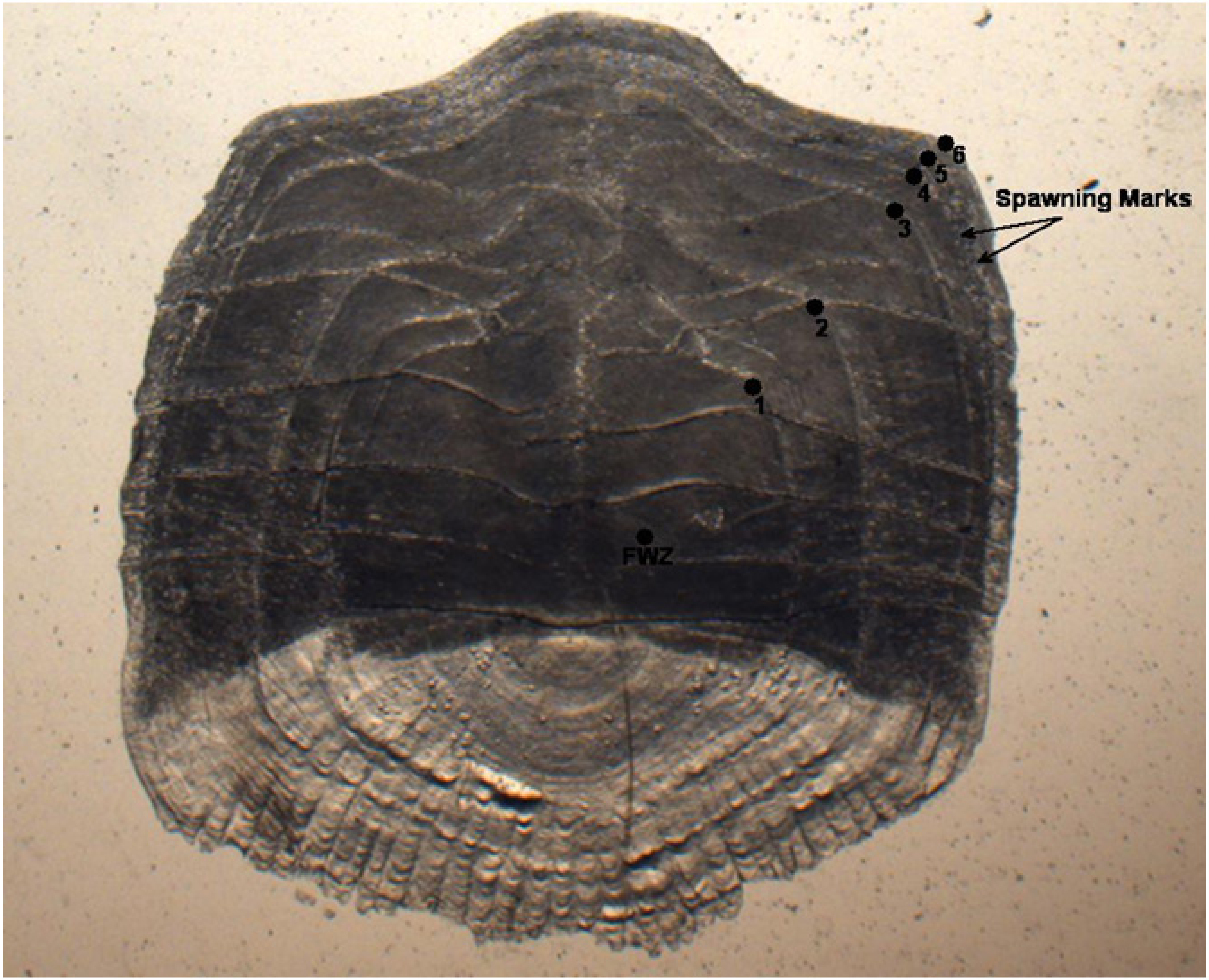


Figure 12. This 6-year-old blueback herring has spawning marks at the fourth and fifth annuli.

American Shad
Alosa sapidissima

American shad are anadromous fish which are most frequently sampled in rivers during their spawning migration. Most fish sampled are four to eight years old.
Scales and otoliths are collected. Otoliths yield better ageing precision than scales, and are preferred when available. Scales can be partially resorbed when fish enter fresh water to spawn (Elzey et al. 2015). The marks left by the resorbtion can be used to identify spawning checks (Cating 1953). Spawning check data are valuable to management, therefore our lab processes scales as well as otoliths.

Sample Collection

- Each fish is given a unique sample ID number (river, year, fish number).
- Length (fork and total), weight, species, sex, collection date, and sample ID number are recorded.
- Otolith extraction:
  - o Using a scalpel or knife, the top part of the head is excised to expose the brain cavity.
  - o The skull removal begins at the back of the skull slicing forward through a point just above the eyes (Figure 13a).
  - o Using forceps, the brain matter is removed with a forward scooping motion.
  - o The otic membranes, one on each side, are extracted with fine tipped forceps (Figure 13b-c).
  - o The otoliths are usually found within the otic membrane, but occasionally remain in the bony case where the membrane was removed. A stream of water from a squirt bottle can be used to dislodge them before removal with fine forceps.
  - o Otoliths are rinsed with water and stored dry in labeled microcentrifuge tubes.
  - o For a video of the procedure see: http://youtu.be/wkGeh-q_Jgo?list=UUlqCy8wTkXK_CbKISYkUHxA
- Scale collection:
  - o Scales are collected ventral of the dorsal fin (Figure 14).
  - o The collection area is scraped with a scalpel or knife to remove slime, other fish's scales, and any debris.
  - o 20-30 scales are collected with a clean knife or scalpel and placed into corresponding envelope.

Otolith Preparation and Ageing

- Preparation
  - o Water is used to clean the otoliths if needed.
- Age Interpretation
  - o Otoliths are immersed sulcus side down in mineral oil, on a black background, and viewed under a stereomicroscope with reflected light (Figure 15).
  - o Annuli are defined as continuous hyaline (dark) bands with no breaks (Figures 16-18).
  - o False annuli typically are not continuous, appear outside of expected areas, lack a defined edge, or connect with true annuli (Figures 16 and 18).
  - o Annuli are counted from the focus outward along the pararostrum or antirostrum.
  - o Avoid using otoliths that are entirely or partially crystallized (Figure 19).

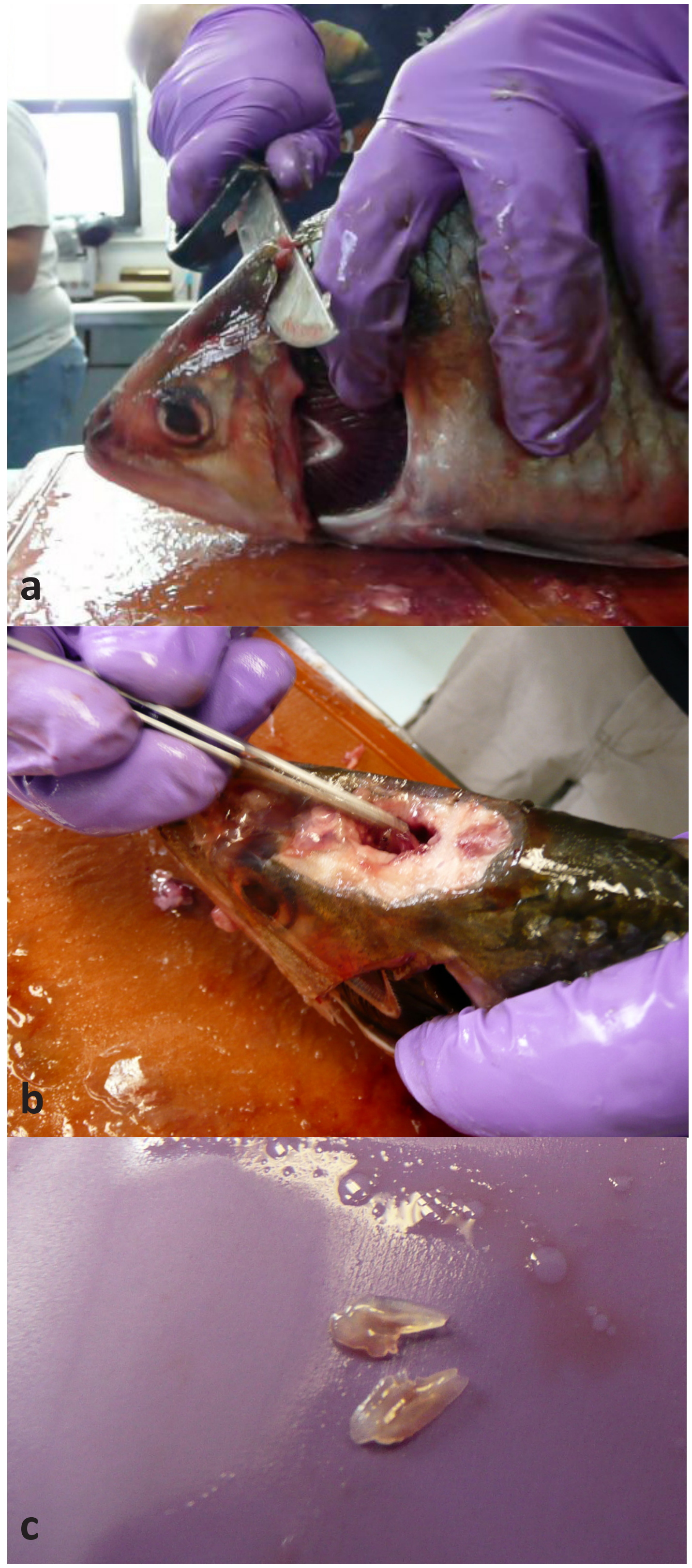


Figure 13a-c. Otolith extraction procedure for alosids, as seen on an American shad. From left to right, (a) opening the skull, (b) removing brain matter, and (c) extracted otoliths.

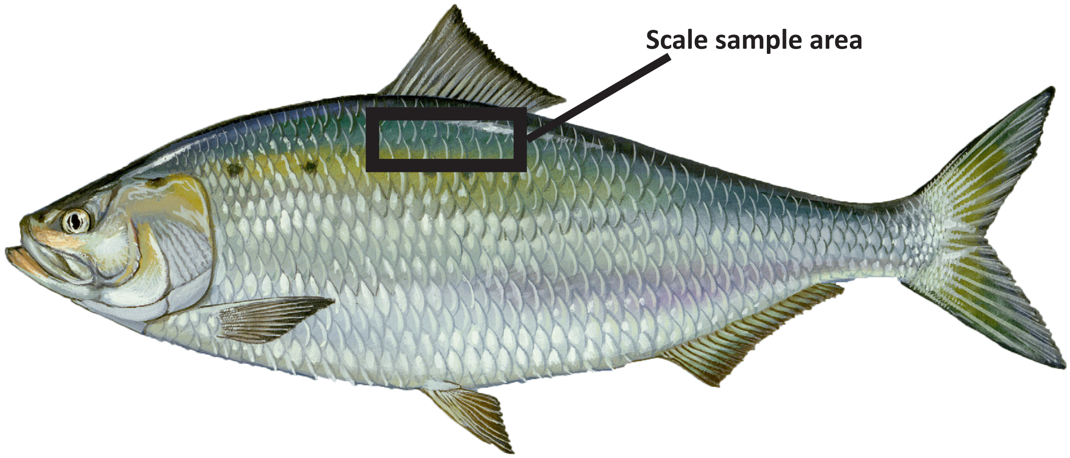


Figure 14. Scale sample area for an American shad, indicated by the rectangle.

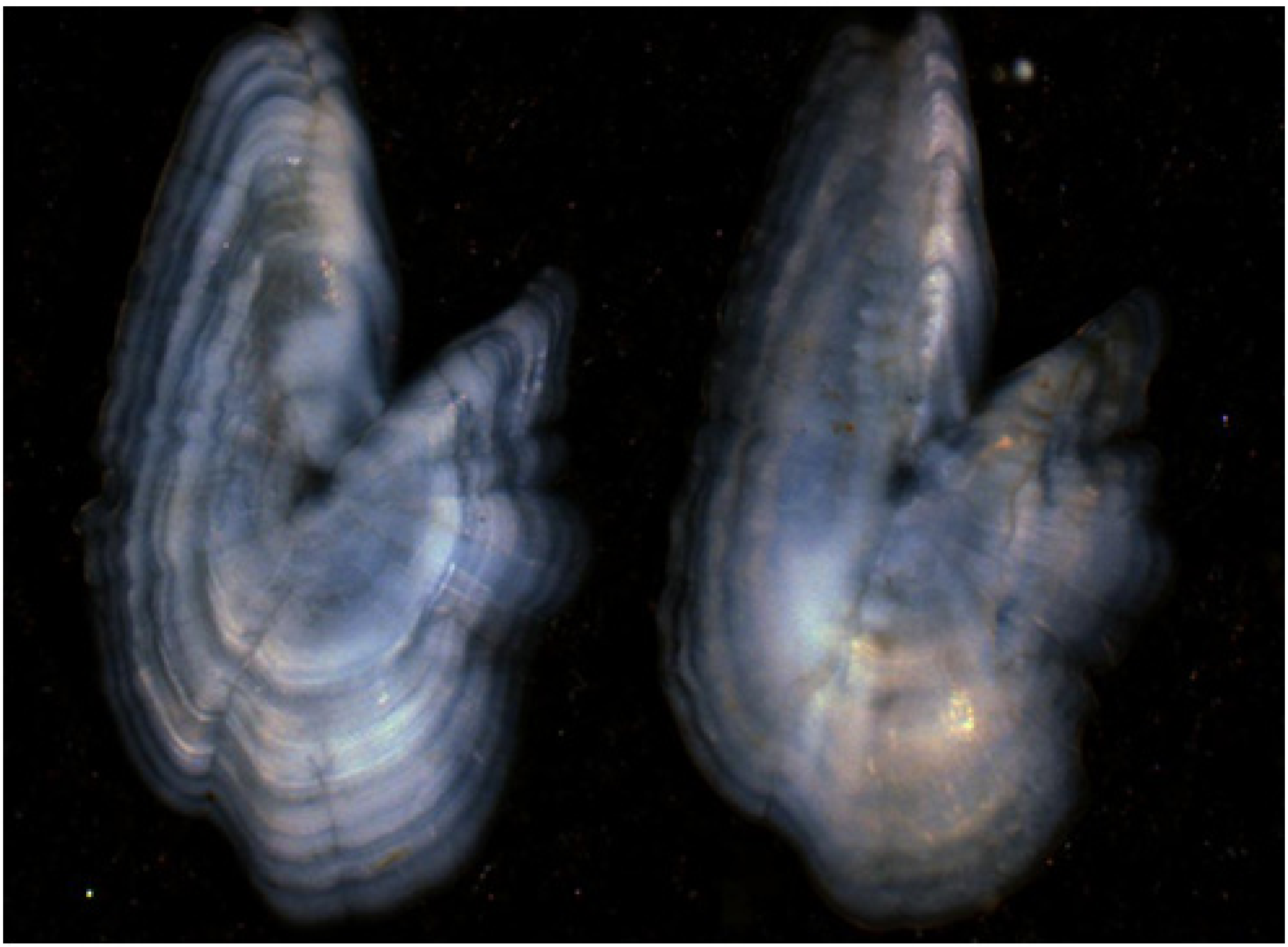


Figure 15. The otolith on the left is the correct side up, while the otolith on the right is sulcus side up. Note how the annuli on the left otolith are more visible.

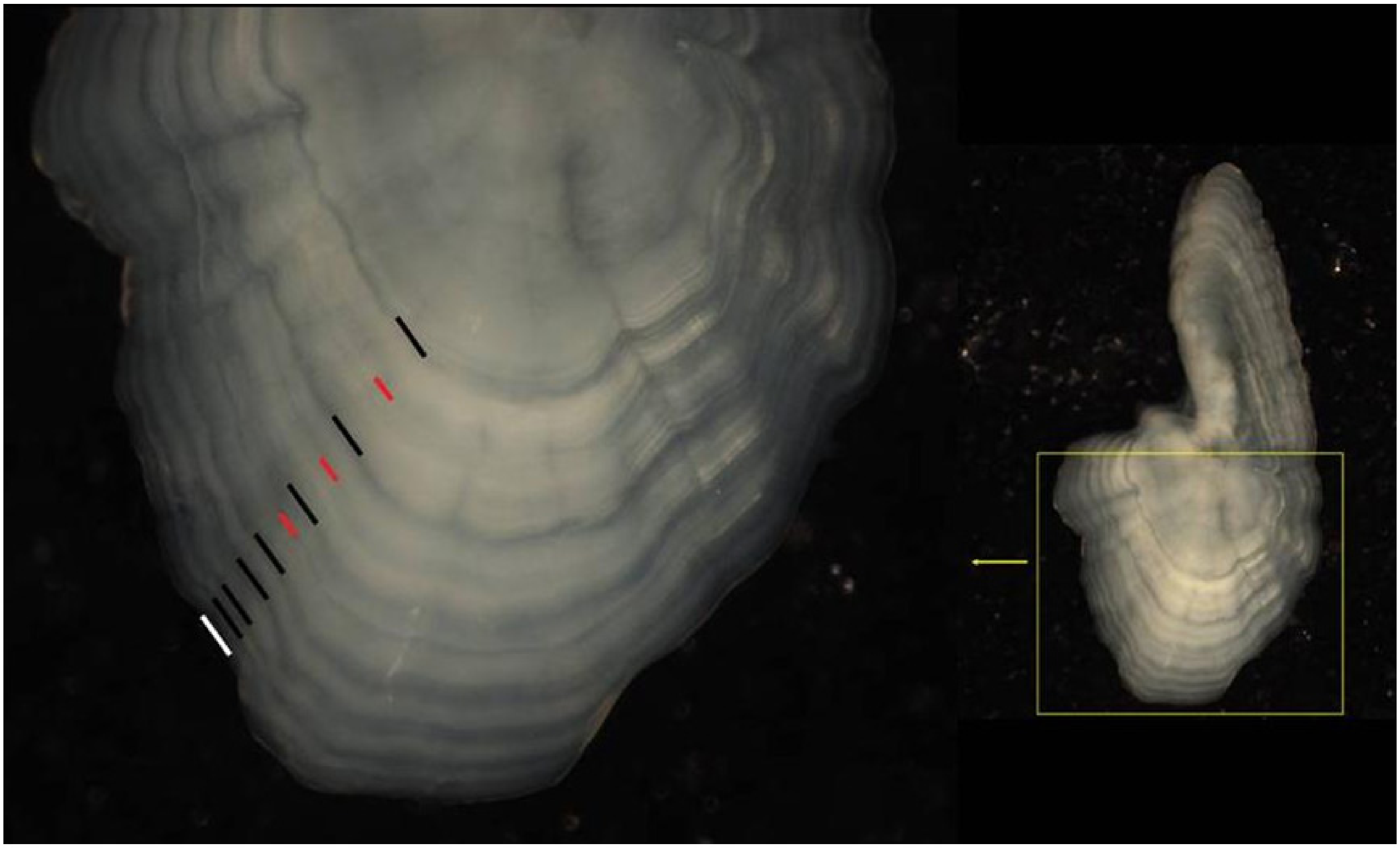


Figure 16. The pararostrum of this 8-year-old American shad is magnified. True annuli are marked with black lines; red lines mark false annuli and the white line marks the margin. The margin is considered the 8th annulus because this fish was captured in spring.

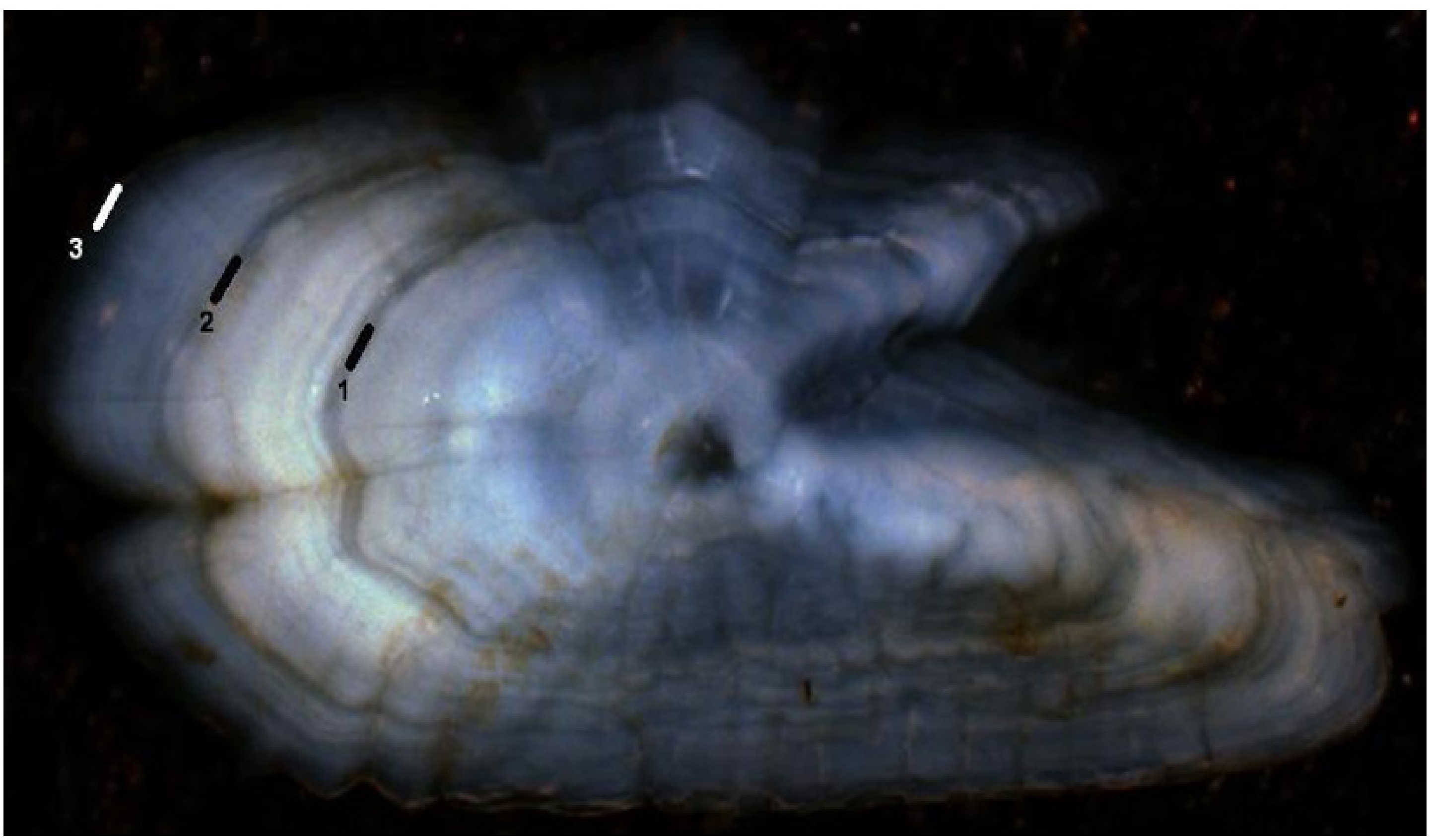


Figure 17. The annuli on this 3-year-old American shad otolith are marked with bars. The margin is counted as the final annulus.

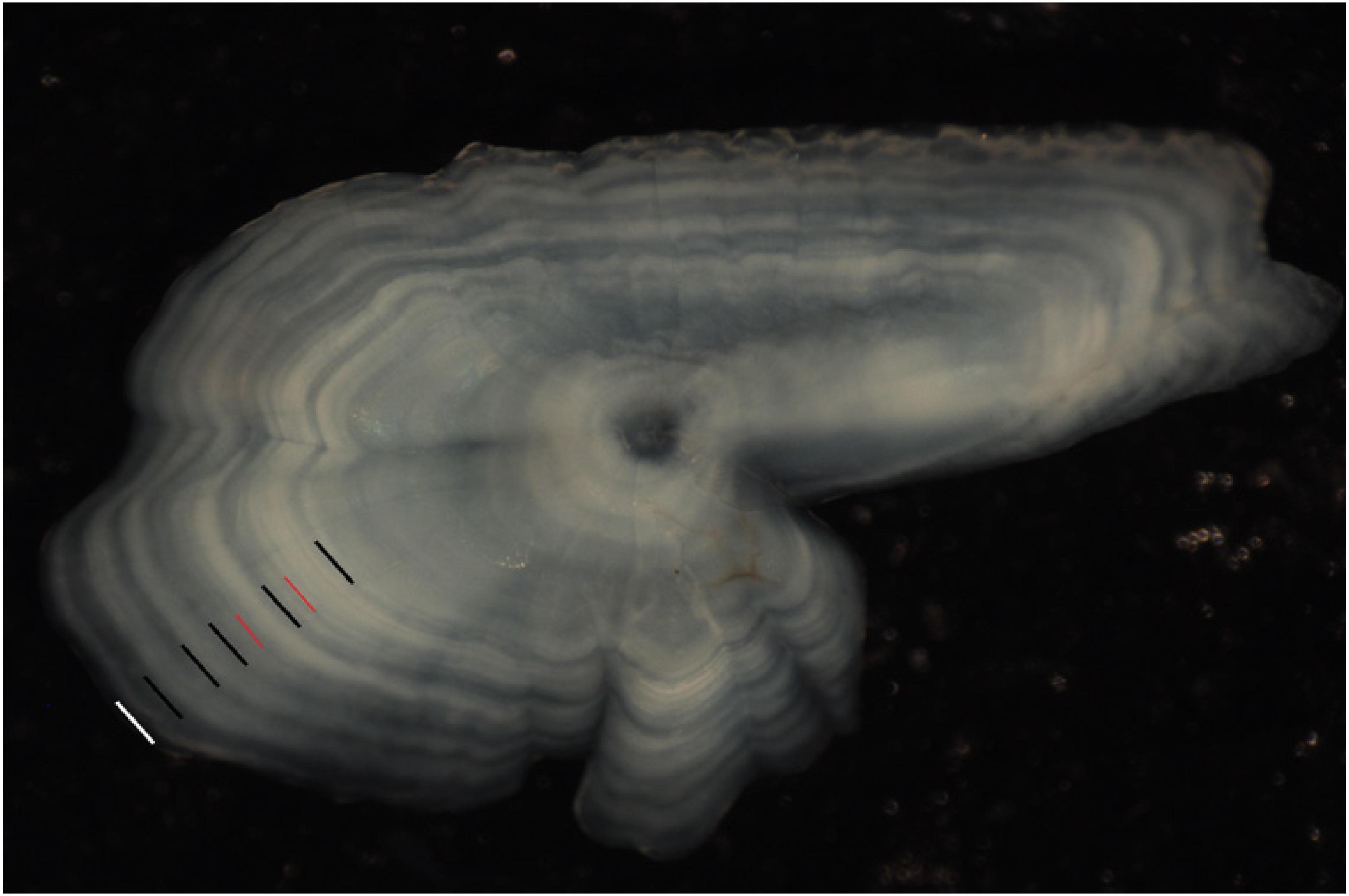


Figure 18. The 5 annuli on this 6-year-old American shad are marked in black along the paraostrum. False annuli are marked with red lines and the margin is marked with white. We count the margin as the 6th annulus because this fish was captured in the spring.

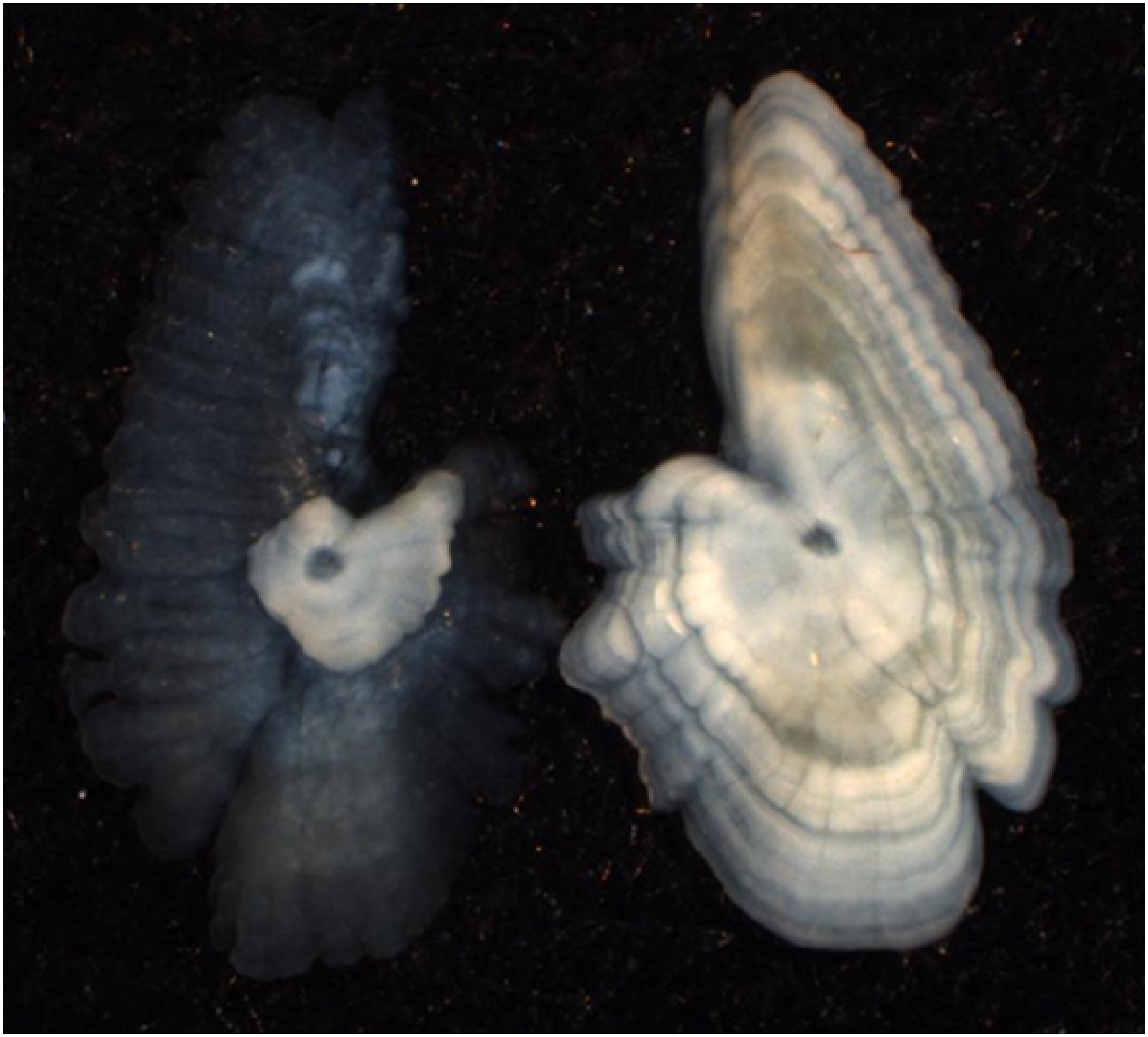


Figure 19. These otoliths are from the same fish. The otolith on the left is crystallized.

Scale Preparation and Ageing

- Preparation
  - o Three or four readable scales are selected per fish, avoiding regenerated and irregularly shaped scales.
  - o A 5% pancreatin solution is prepared and the scales are cleaned in a sonic cleaner following the method outlined by Whaley (1991).
  - o The pancreatin is drained from scales using a small mesh sieve (The pancreatin can be saved and used several times).
  - o The scales are then placed in a dish of clean water, wiped clean and dried with paper towels as needed.
  - o The dry scales are placed between two glass slides labeled with the appropriate ID number.
- Age Interpretation
  - o Scales are viewed using transmitted light with image processing software and a camera on a macro mirror stand.
  - o The mirror and lighting are adjusted so annuli can be viewed crossing over the baseline.
  - o Annuli appear as continuous strong bands that cross the transverse grooves and continue past the baseline.
  - o Annuli are counted from the center outward (figure 20).
  - o The first dark band is usually the freshwater zone (Figure 20).
  - o The first annulus is frequently weak and doesn't always follow the annulus criteria.
  - o The edge is counted as the last annulus, if captured in spring.
  - o False annuli will not cross over the baseline, cannot be followed throughout the scale, or cannot be seen on every scale (Figure 21).
  - o On older fish, annuli can become crowded together at the edge of the scale, but will separate beneath the

baseline. These should be counted as separate annuli (Figures 22 and 23).

o Scale resorbtion during spawning can remove parts of previous annuli. These annuli will still separate beneath the baseline and should be counted separately (Cating 1953).

o Spawning marks are identified as annuli that appear fuzzy and jagged above the baseline or that show that they've resorbed over another annulus above the baseline (Figures 22 and 23).

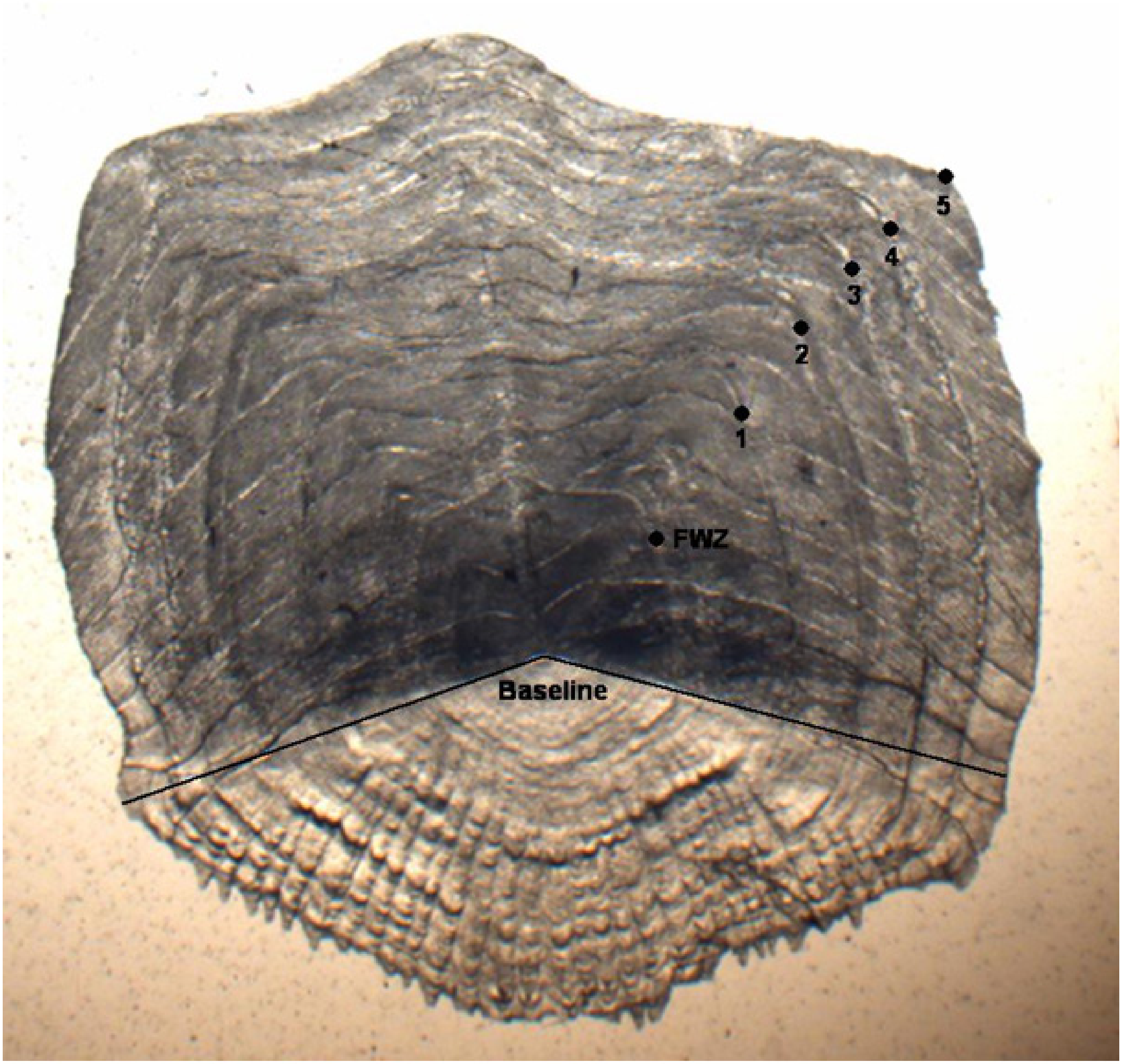


Figure 20. Scale from a 5-year-old American shad with annuli, baseline, and fresh water zone marked.

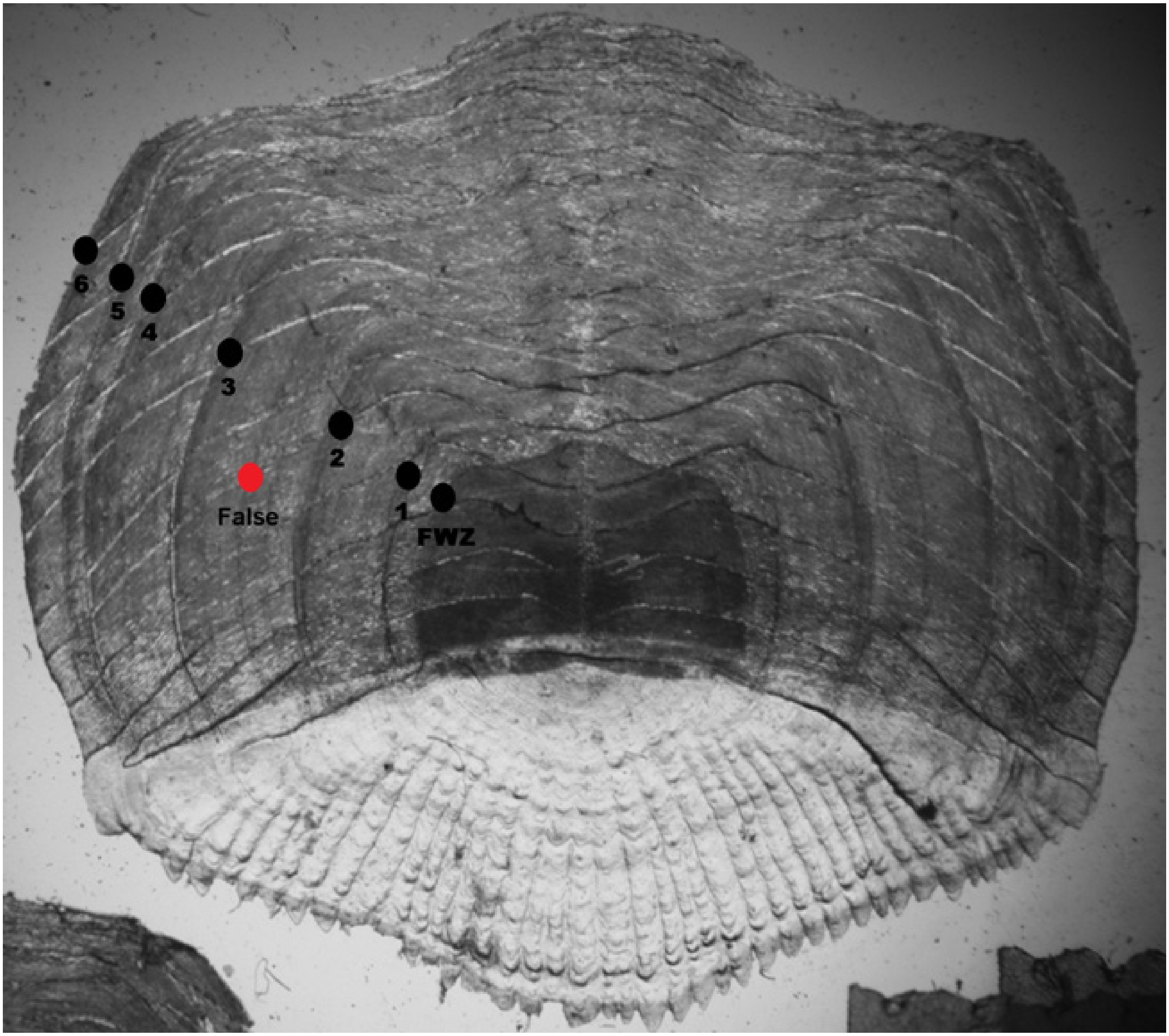


Figure 21. Scale from a 6-year-old American shad with a false annulus.

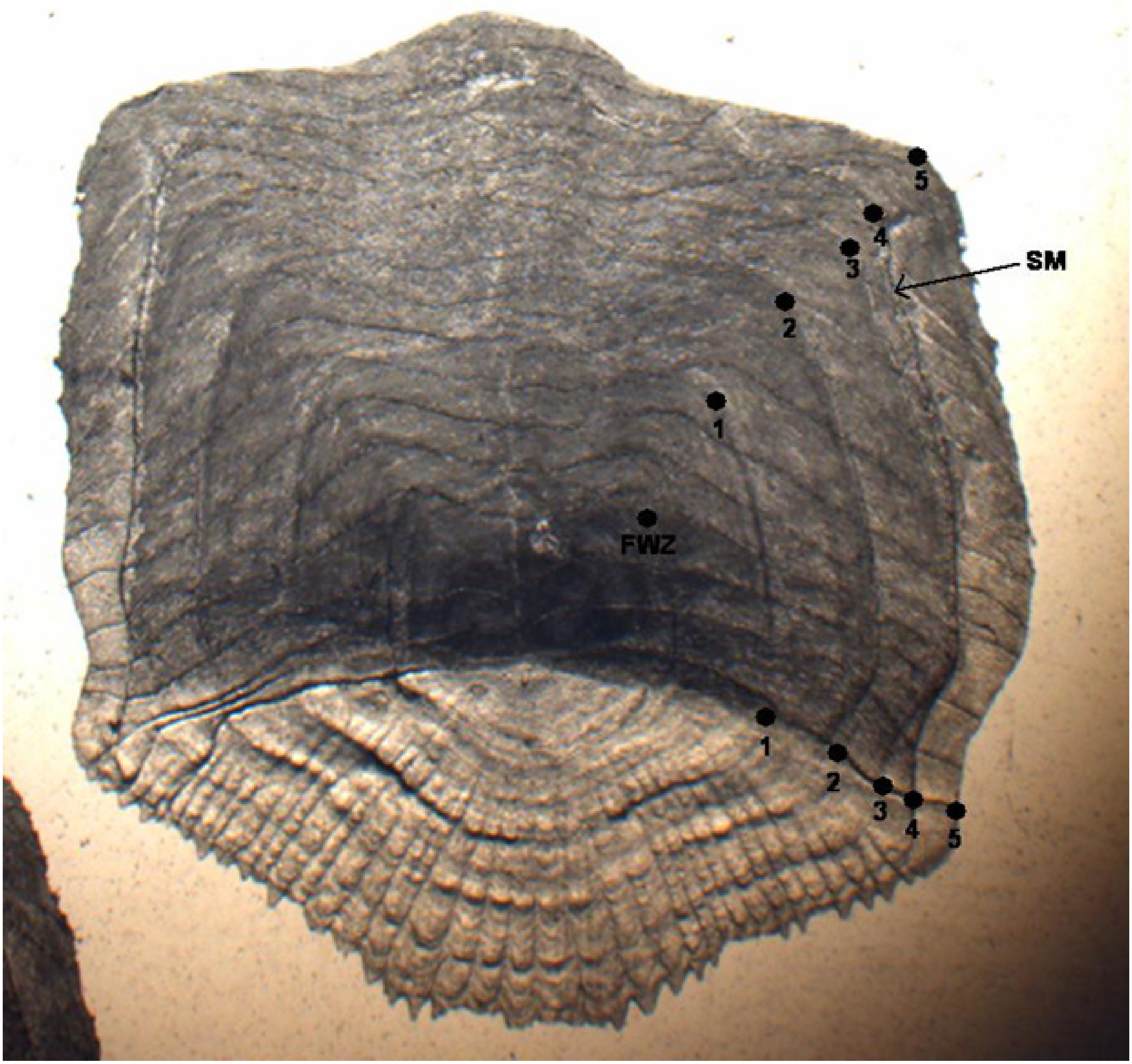


Figure 22. A 5-year-old American shad scale shows a spawning mark (SM) at age 4. Note how the 4th annulus is jagged and overlaps the 3rd.

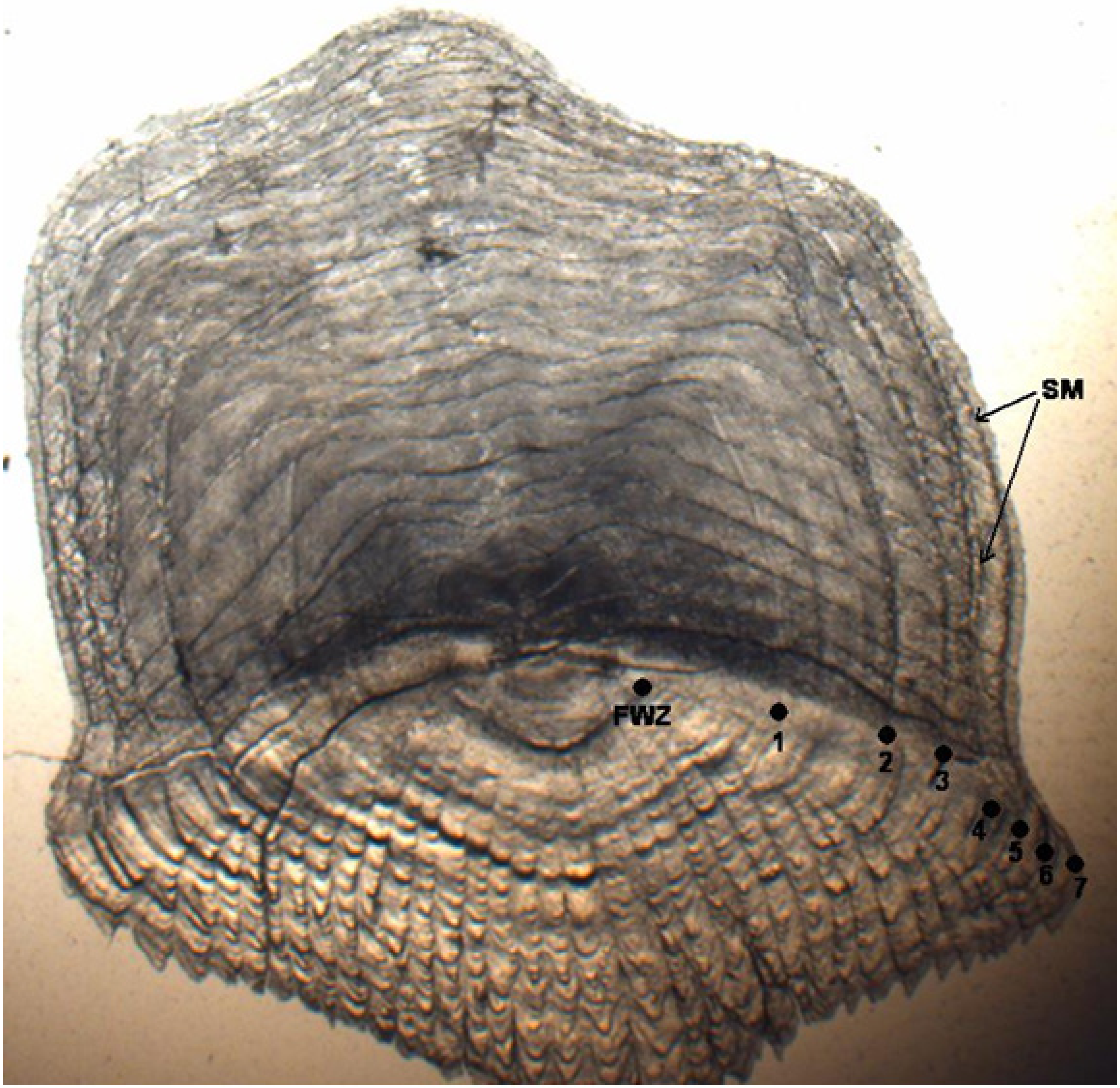


Figure 23. A 7-year-old American shad shows two spawning marks (SM). Note how the annuli near the edge are crowded together above the baseline and not below it, causing the classic "bell" shape.

Black Sea Bass
Centropristus striata

Black sea bass otolith samples are collected through the Massachusetts Division of Marine Fisheries resource assessment trawl surveys in the spring and fall. Scale samples are collected via port sampling of the commercial fishery. Most fish aged are between one and five years old.

Sample Collection

- Length, sex, capture date, location, and sample ID number are recorded on the collection envelope and data sheet.
- Scale collection
  - o Scales are collected from behind the pectoral fin.
  - o The collection area (Figure 24) is scraped with a scalpel or knife to remove slime, other fishs' scales, and any debris.
  - o Scales are collected with a clean knife or scalpel and placed into corresponding envelopes.
- Otolith extraction
  - o Using a sharp knife, a vertical cut is made through the forehead just behind the pre-opercula.
  - o A second cut is made horizontally, starting just above the eye to connect with the first cut.
  - o The otoliths are removed using forceps.
  - o Otoliths are rinsed when possible and stored dry in labeled envelopes.

Scale Preparation and Ageing

- Preparation
  - o Up to six scales, where the radii come to a clear point at the focus, are selected (Figure 25).
  - o Regenerated scales (Figure 26) are avoided.
  - o Scales from fish 180 mm and smaller (total length) are mounted between two slides.
  - o Scales from fish greater than 180 mm are pressed into acetate.
  - o Scales are placed on a sheet of acetate, rough (distal) side down.
  - o Press samples at 12,000 lb. for 3 minutes in a press heated to 100^{o}C.
  - o After samples cool, the scales are pulled from the acetate.
  - o Each sample is labeled with its corresponding sample ID number.
- Age Interpretation
  - o Scales and impressions are viewed using transmitted light with image processing software and a camera on a macro mirror stand.
  - o The mirror and lighting are adjusted so the annuli can be viewed cutting over near the baseline (Figures 25 and 27).
  - o Annuli are counted from the middle outward.
  - o Annuli appear as a disturbance in the circuli throughout the anterior portion of the scale and cut over near the baseline (Figures 25 and 27).
  - o On older fish, annuli will become crowded near the edge of scales.
  - o Fish captured in between January 1 and June 30 will have the edge counted as the last annulus.
  - o Fish captured between July 1 and December 31 will have growth beyond the last visible annulus, but the edge is not counted.

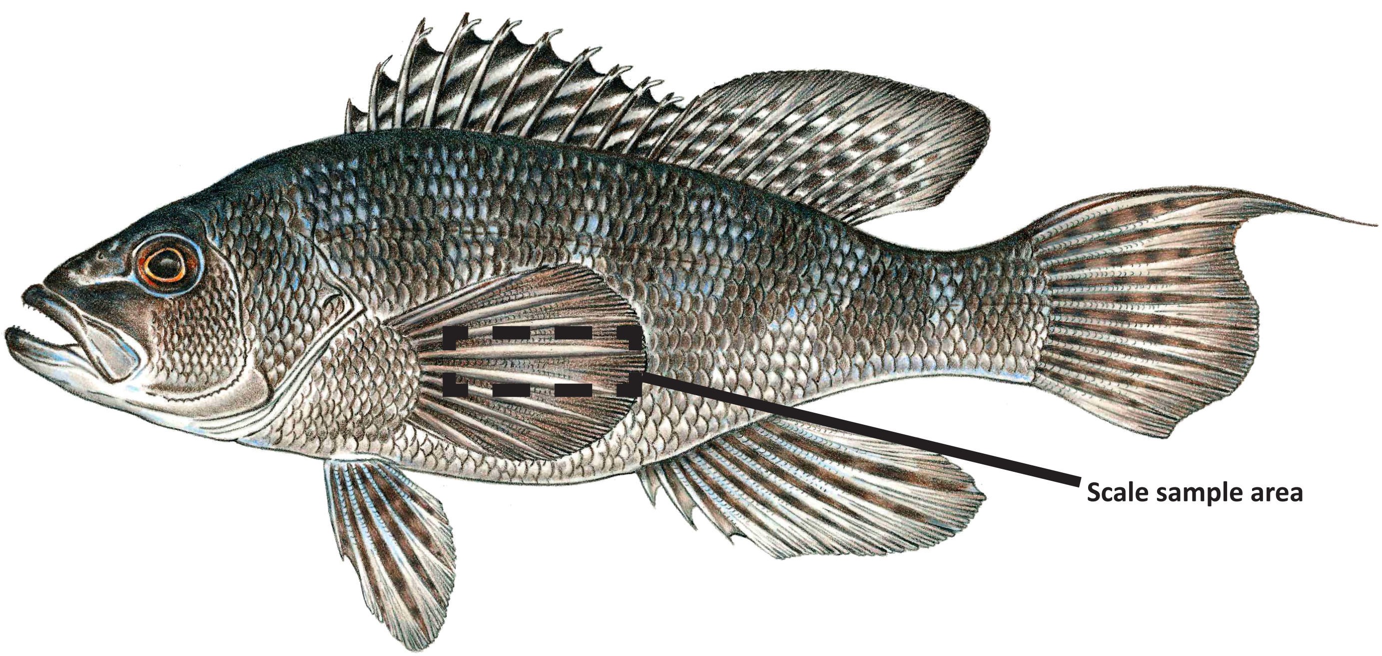


Figure 24. Scale sample area on a black sea bass is located behind the pectoral fin, as indicated by the dashed rectangle.

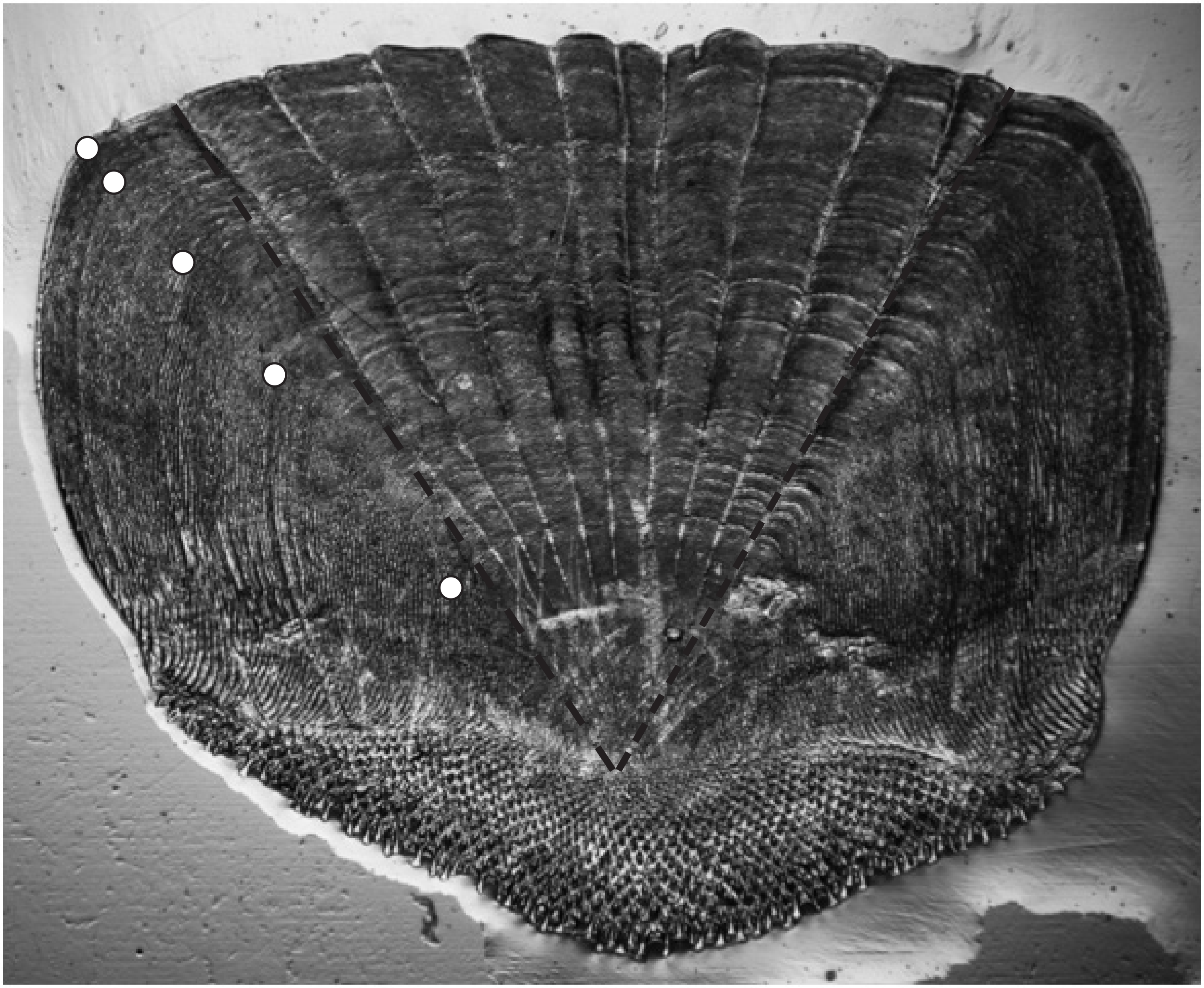


Figure 25. This scale from a 5-year-old is appropriate for ageing. Note how the radii form a V-shape (dashed line). The edge is counted as an annulus because this fish was captured in the spring.

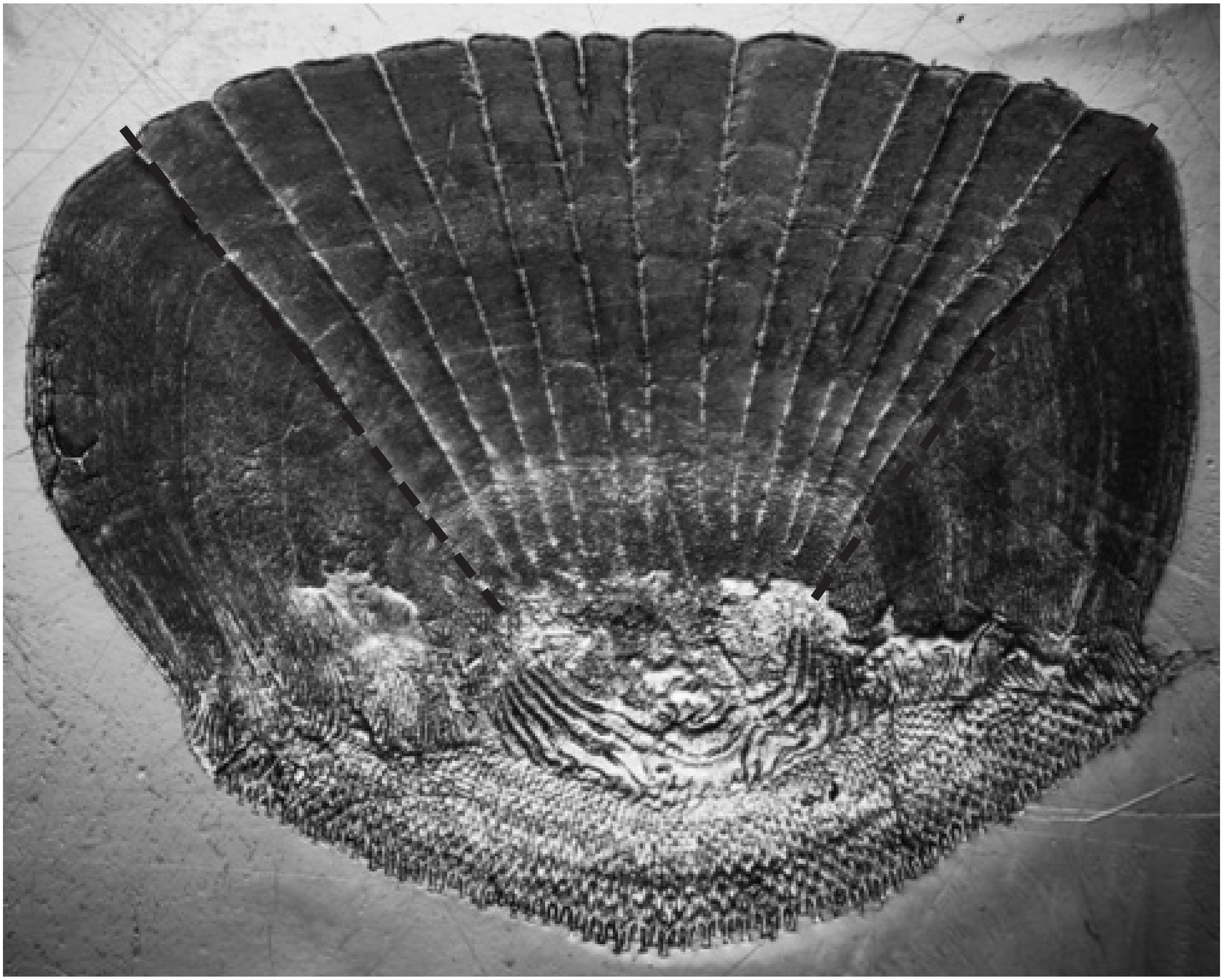


Figure 26. This scale has a regenerated center and is therefore not appropriate for ageing.

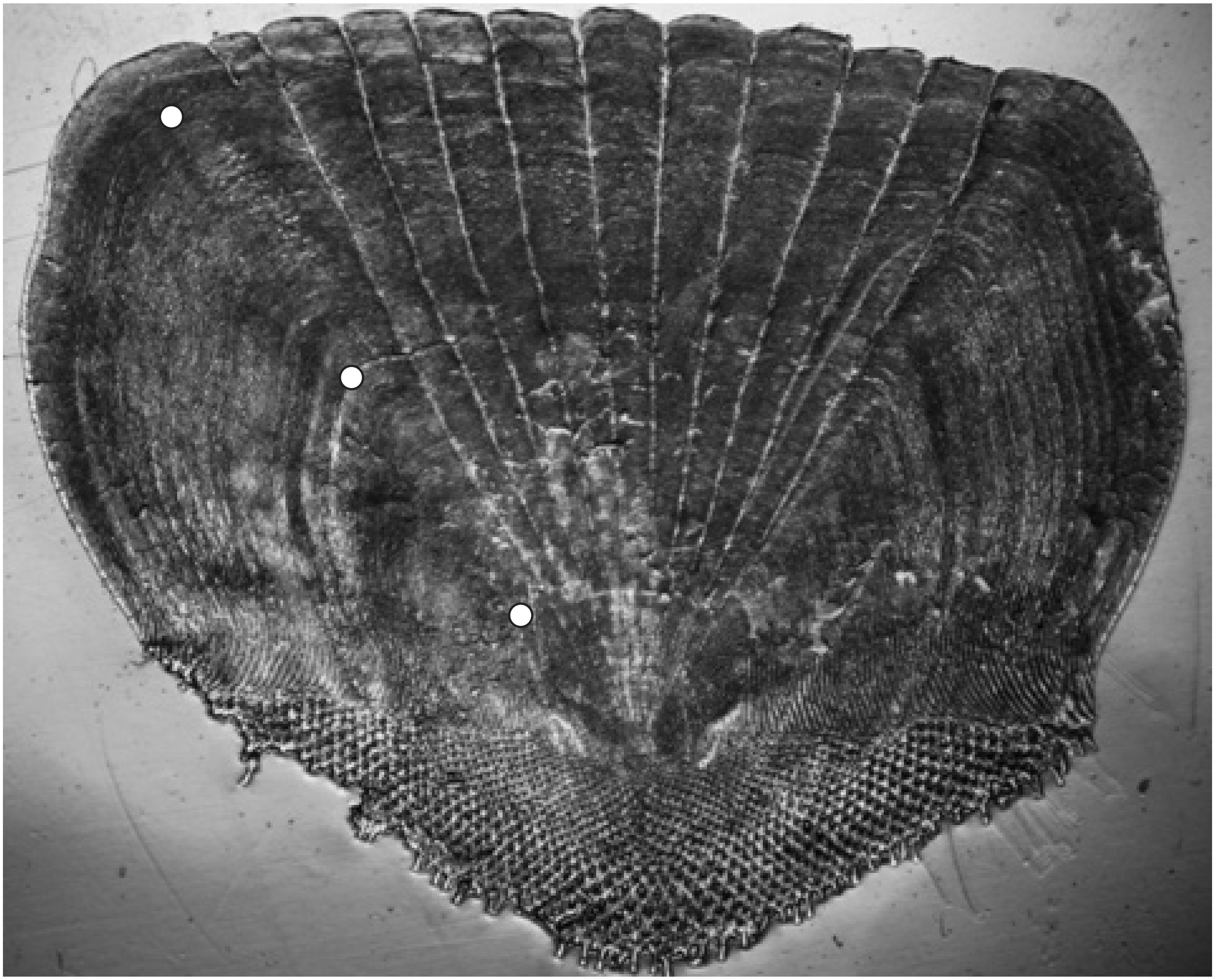


Figure 27. This scale is from a 3-year-old fish captured in the fall, therefore we do not count the edge as an annulus.

Whole Otolith Preparation and Ageing

- Otoliths are stored dry in envelopes labeled with length, weight, sex, location, and ID number.
- Water is used to clean off any dried blood.
- Otoliths are immersed in mineral oil, sulcus down, on a black background and viewed under a stereomicroscope with reflected light.
- Annuli are counted outward from the core (Figure 28).
    - o Annuli are defined as continuous hyaline (dark) bands with no breaks (Figure 28).
    - o False annuli typically are not continuous, lack a defined edge, or split from true annuli (Figure 29).
    - o Fish collected between January 1 and June 30 will have an annulus forming at the margin, therefore the edge will be counted as the last annulus (Figure 28).
    - o Fish collected between July 1 and December 31 will have growth after the last annulus, therefore the edge will not be counted as the last annulus (Figure 29).
    - o Otoliths aged as six or older are sectioned to confirm age (Figure 30).
- An image of the whole otolith is taken prior to sectioning.
- Otoliths are stored dry to avoid clearing.

Otolith Section Preparation and Ageing

- Otoliths are affixed sulcus side up to a glass slide with Crystal Bond.
- An IsoMet low speed saw is used to cut through the otolith core to create transverse thin-sections approximately 0.4 mm thick (Figure 30).
- Minimal weight is used on the saw to keep from dislodging the otolith from the slide.
- The otolith sections are affixed to a pre-labeled slides using Flo-Texx liquid cover slip.
- Care is taken to remove any air bubbles that may form.
- Sections are viewed through a compound scope at 100x using transmitted light.
- Annuli are counted from the core outward along the dorsal side of the sulcal groove (Figure 31).
- Care is taken to make sure all annuli are counted within the same plane of focus.
- A good section will have a V-shaped sulcus that extends to the core (Figure 31).
- A poor quality section will have a tornado shaped sulcus that might not extend to the core.
- Occasionally, one side of a section looks better than the other; when age determination is difficult, the slide is examined from the back side.

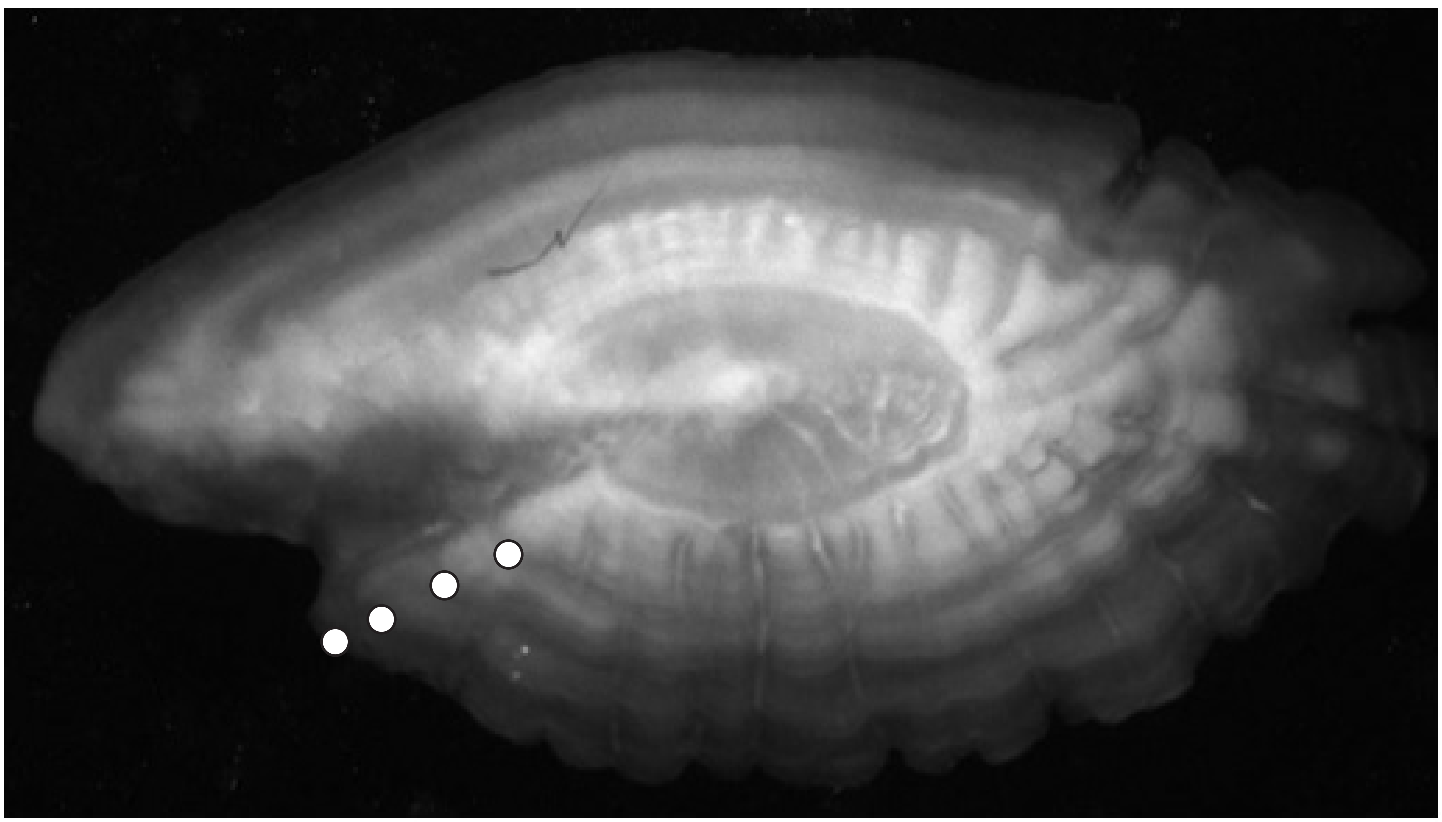


Figure 28. This is a 4-year-old otolith with each annulus marked with a dot. This fish was captured in the spring, therefore the edge is counted as an annulus.

Figure 29. This is a 3-year-old with each annulus marked with a white dot. The red dot marks a false annulus. Note how the false annulus is not continuous. This fish was captured in the fall, therefore the edge is not counted as an annulus.

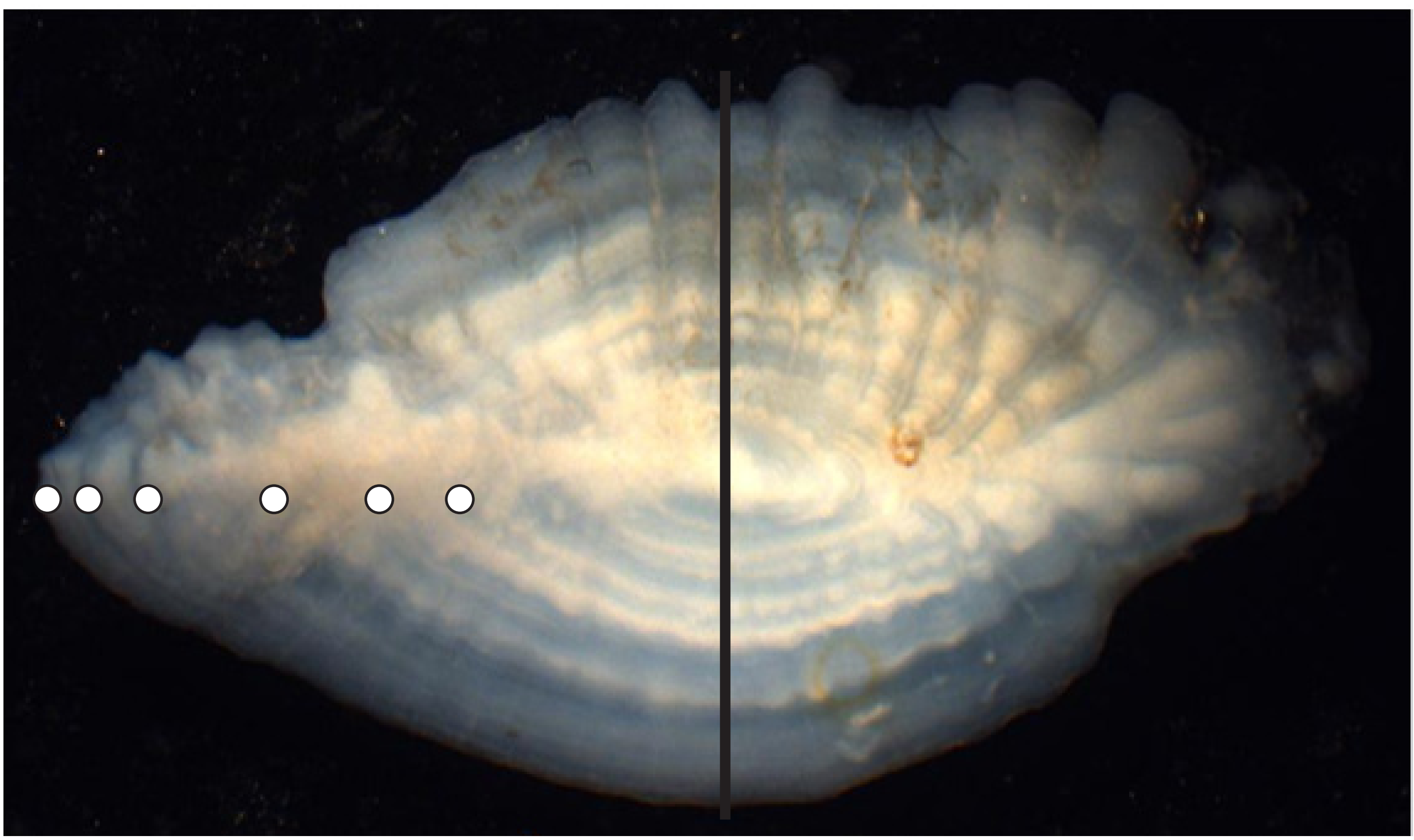


Figure 30. This is a 6-year-old fish that will be sectioned to confirm age. This fish was captured in the spring, so the edge will be counted as the last annulus. A line marks the ideal sectioning axis.

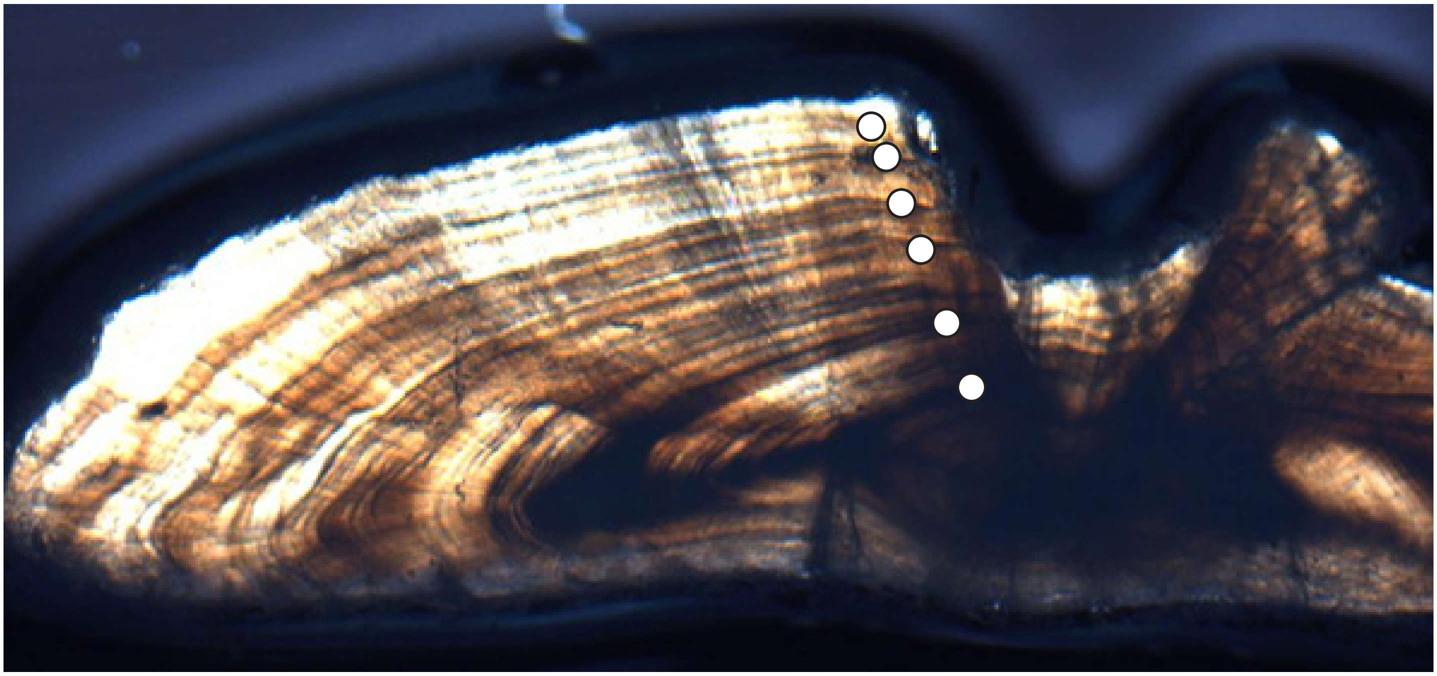


Figure 31. This is a section of a 6-year-old. Note that the sulcus is V-shaped and ends near the core. The annuli are marked with white dots along the ideal ageing axis. The edge is not counted as an annulus because this fish was captured in the fall.

Bluefish
Pomatomus saltatrix

Bluefish samples are collected via hook and line throughout the summer. Bluefish age data are combined among states by the Atlantic States Marine Fisheries Commission (ASMFC). The recommendations of a recent ASMFC bluefish ageing workshop were that all states with more than 5% of the total harvest provide a minimum of 100 ages, spread across the age range and capture dates for the state (ASMFC 2012). Most fish aged in Massachusetts range from two to seven years old.

Sample Collection

- Length, sex, capture date, location, and sample ID number are recorded on a data sheet.
- Otolith Extraction:
  - o A hacksaw is used to make a vertical cut through the forehead just behind the pre-opercula.
  - o A second cut is made horizontally, starting just above the eye to connect with the first cut.
  - o Otoliths are removed using forceps.
  - o When possible, otoliths are rinsed and stored dry in labeled microcentrifuge tubes.

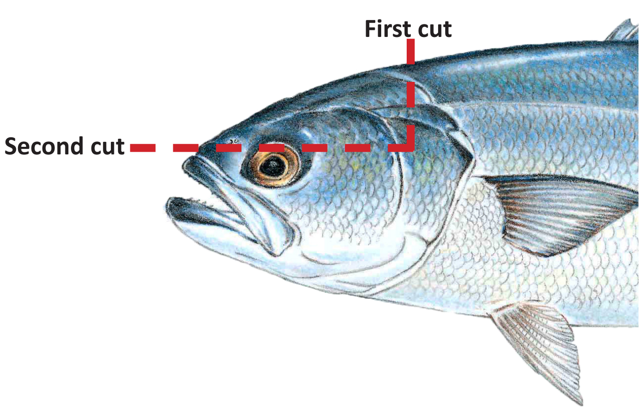


Figure 32. Location of proper bluefish otolith extraction cuts.

Otolith Preparation and Ageing

- Preparation
  - o An empty porcelain tray is heated in a 400ºC furnace.
  - o The tray is removed from the oven and the otoliths are placed into numbered tray wells.
  - o A properly heated tray will hold enough heat that the otoliths will "bake" quickly without needing to return the try to the oven.
  - o When otoliths turn golden "caramel" brown they are removed them from tray.
  - o A pencil is used to mark the otolith transversely through the core.
  - o Otoliths are then embedded in two-part epoxy in silicon bullet molds.
  - o An IsoMet low speed saw is used to cut through the otolith core to create transverse thin-sections approximately 0.4 mm thick (Figures 33 and 34).
  - o The thin-sections are affixed with Flo-Texx liquid cover slip to slides labeled with their corresponding sample ID numbers.
  - o Care is taken to remove any air bubbles that form in the Flo-Texx.

- Age Interpretation
  - o Sections are viewed under transmitted light with compound scope at 100x.
  - o Annuli are counted from the core outward along the sulcal groove (Figure 35).
  - o Crenellation formation can help identify annuli (Figure 35).
  - o Care is taken to ensure all annuli counted are in the same plane of focus.
  - o A properly cut otolith will yield a section with a V-shaped sulcus that extends to the core (Figure 35).
  - o A poor quality section will have a twisted tornado shaped sulcus that might not extend to the core (Figure 36).
  - o Poorly cut sections may look better from the alter-

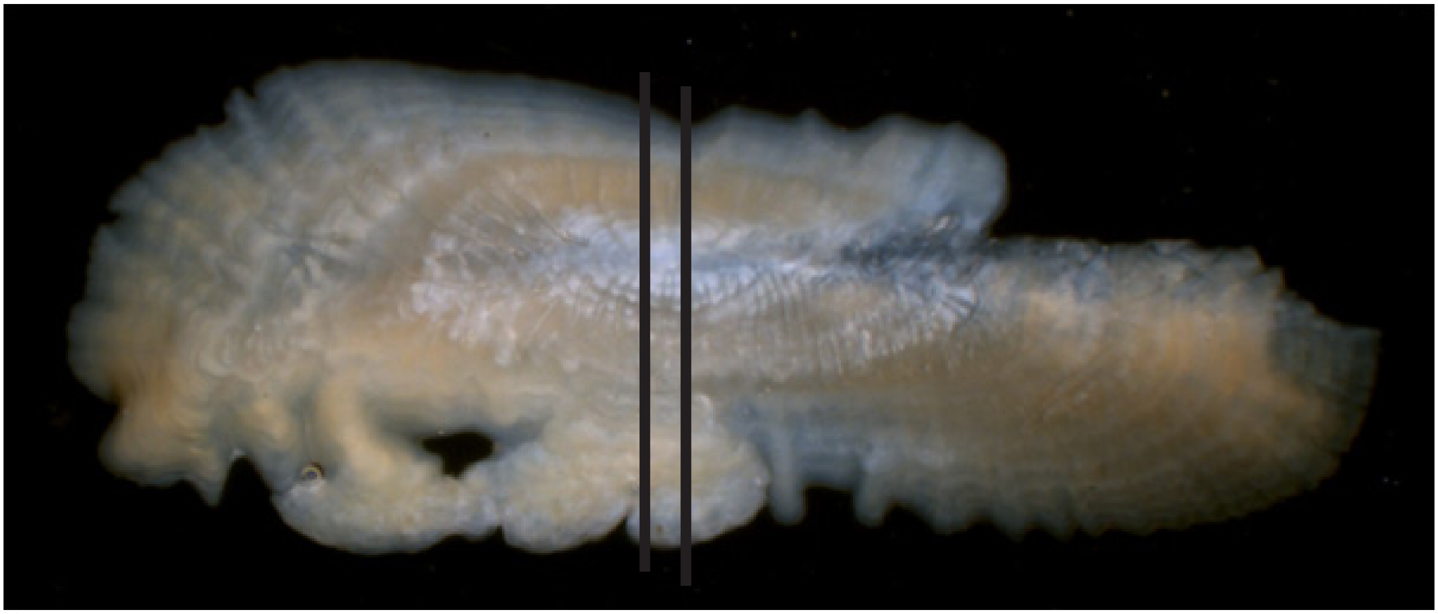


Figure 33. The proper cutting asix for a thin transverse section on a bluefish otolith.

nate side.

o Fish captured in between January 1 and June 30 will have the edge counted as the last annulus.

o Fish captured between July 1 and December 31 will have growth beyond the last visible annulus, but the edge is not counted.

Figure 34. An embedded otolith being sectioned on the IsoMet low-speed saw.

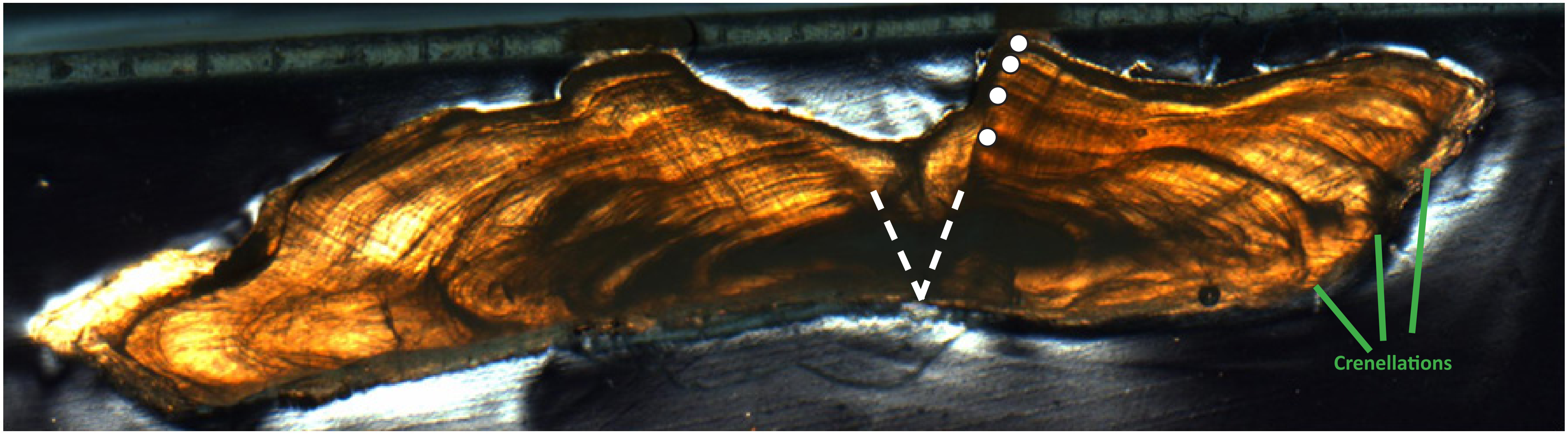


Figure 35. An otolith section from a 4-year-old bluefish. The crenellations along the right edge are great indicators of annulus location. Note how the sulcus comes to a nice V-shape near the core, as indicated with dashed lines. This fish was captured in the spring, therefore the edge is counted as an annulus.

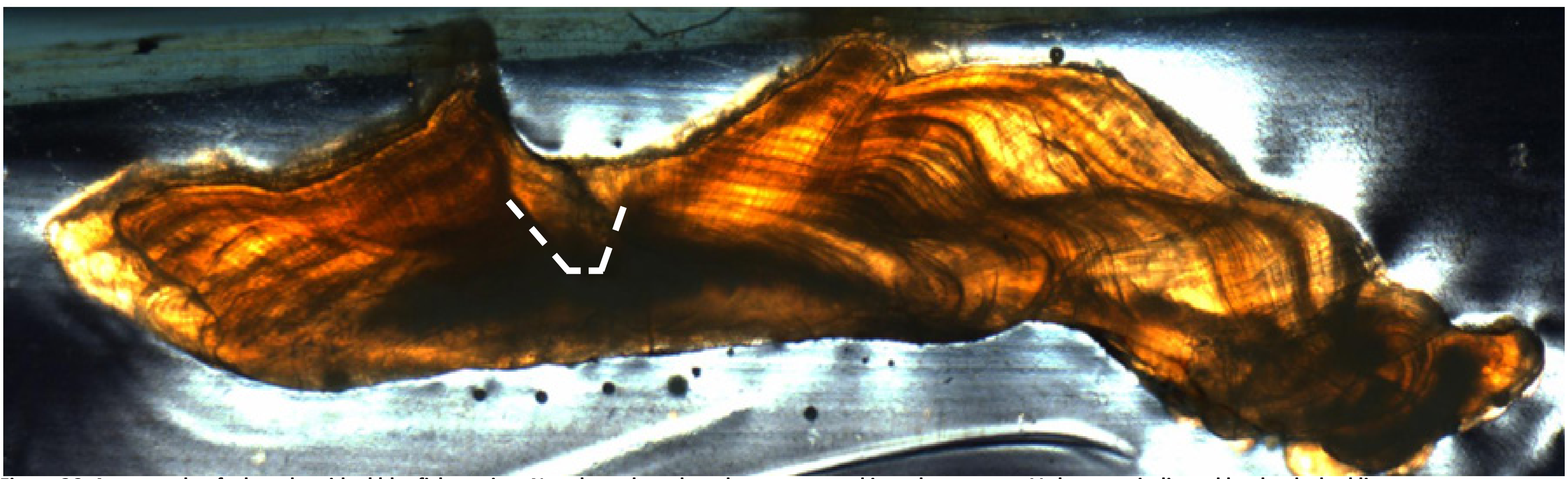


Figure 36. An example of a less than ideal bluefish section. Note how the sulcus does not extend into the core as a V-shape, as indicated by the dashed lines.

Rainbow Smelt
Osmerus mordax

Rainbow smelt are collected with fyke nets in the spring during their spawning migrations into the rivers. Scales are used for age determination because they are easier to read than otoliths. Given that the majority of fish sampled are between one and three years old, the typical problem of under ageing old fish with scales does not apply.

Sample Collection

- Each fish is given a unique sample ID (river, year, and fish number).
- Total length, sex, species, capture date, and sample ID are recorded on envelopes and data sheet.
- Scale collection:
  - o Scales are collected ventral of the dorsal fin as shown in Figure 37.
  - o Mucus and any other debris are removed by scraping with a scalpel or knife from anterior to posterior.
  - o 20-30 scales are collected with a clean knife or scalpel and placed into the corresponding envelope.

Scale Preparation and Ageing

- Preparation
  - o Approximately 20 scales are selected and placed in a microcentrifuge tube.
  - o The tubes are filled with a 5% pancreatin solution (Whaley 1991).
  - o The tubes are floated in a sonic cleaner for approximately 10 minutes (Whaley 1991).
  - o Contents of tube are dumped into a petri dish with clean water.
  - o Scales are wiped, rinsed, and then dabbed on a paper towel.
  - o 16 scales are placed on a slide with the corresponding ID number.
  - o A cover slip is taped over the scales.
  - o Care is taken to avoid contamination between fish.
- Age Interpretation
  - o Scales are viewed with transmitted light on image analysis software.
  - o Regenerated scales are not used for ageing (Figure 38).
  - o Annuli have breakages of circuli on both sides of the scale.
  - o Circuli become more tightly spaced just inside an annulus and more spread out after an annulus.
  - o Annuli can be followed around the entire scale, false annuli cannot.
  - o Annuli will be accompanied by a “shiny line” scar as described by McKenzie (1958).
  - o Annuli will appear on every scale.
  - o The edge is counted as an annulus on fish captured in the spring.
  - o In Massachusetts, circuli will form inside the first annulus.
  - o Circuli around false annuli will not break on both sides (Figure 39).
  - o Older fish may only have one or two circuli between annuli (Figure 40).

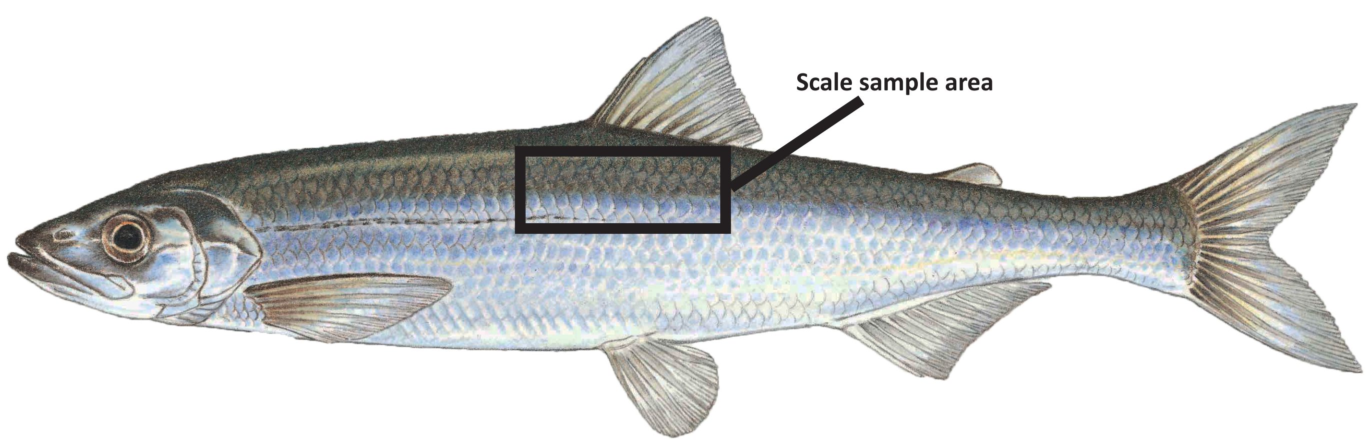


Figure 37. Scale sample area depicted by the rectangle on a rainbow smelt.

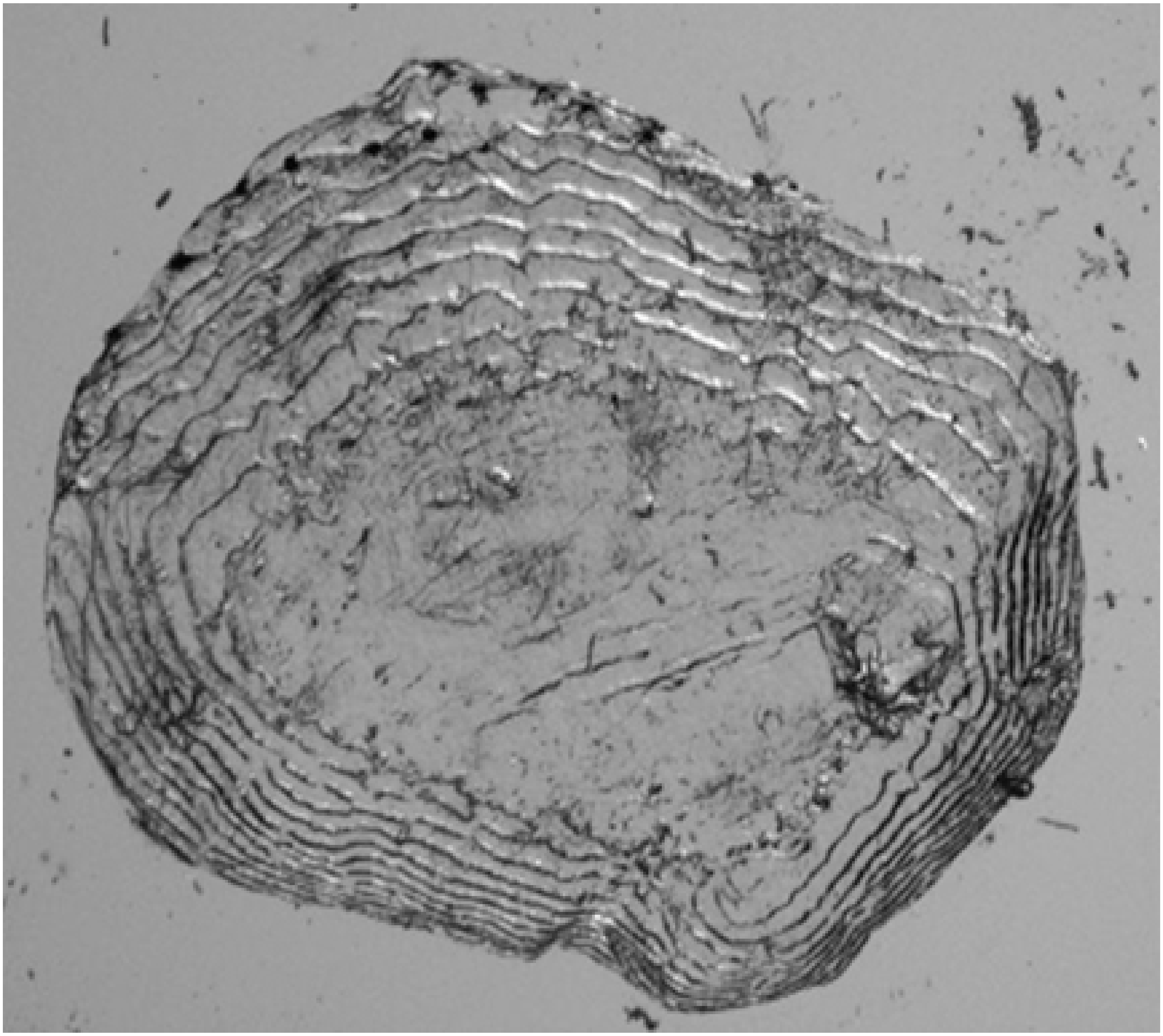


Figure 38. A regenerated smelt scale. Note the lack of circuli near the origin of the scale.

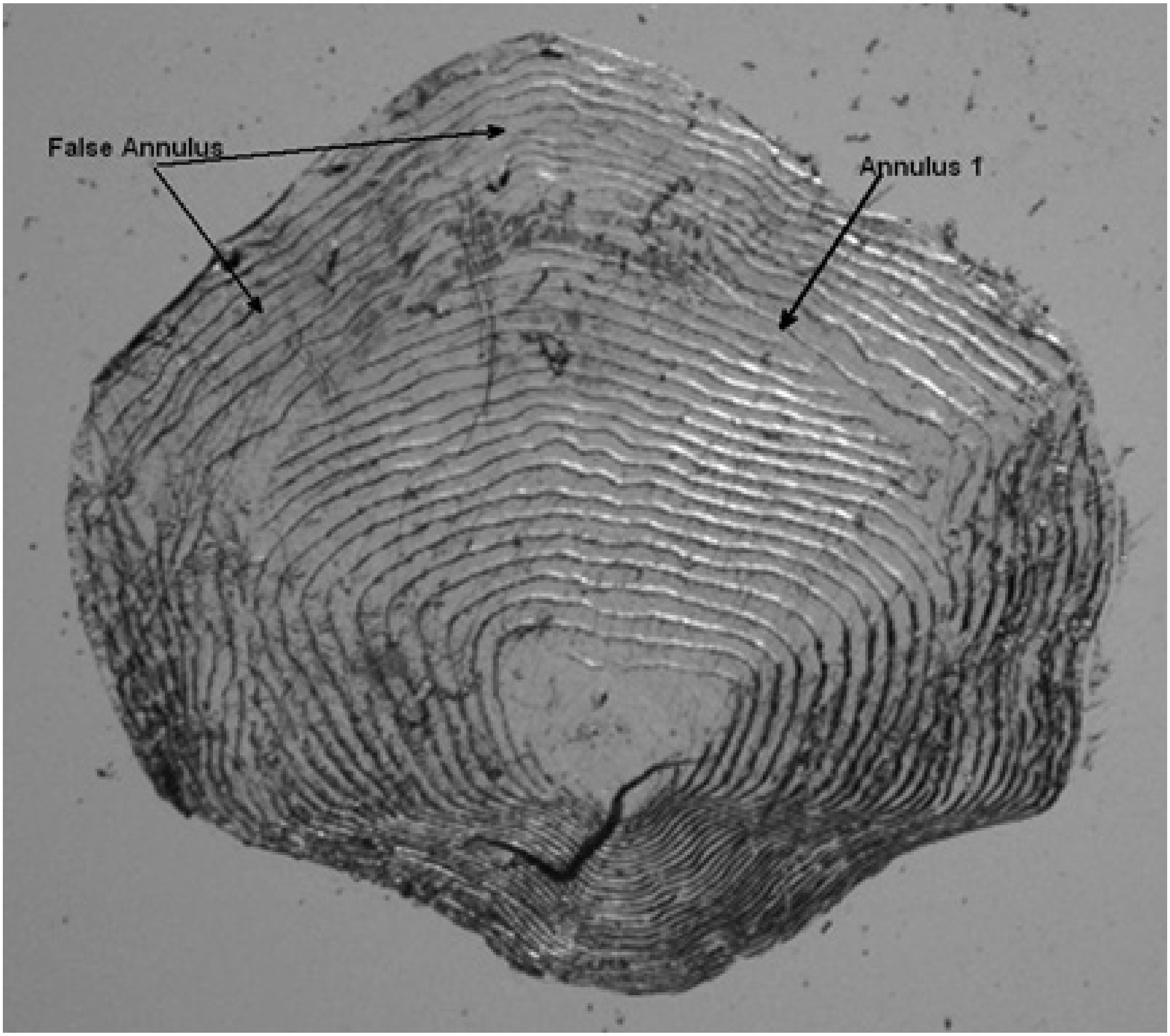


Figure 39. A scale from a 2-year-old rainbow smelt. Note how the circuli around the false annulus only break on the left side.

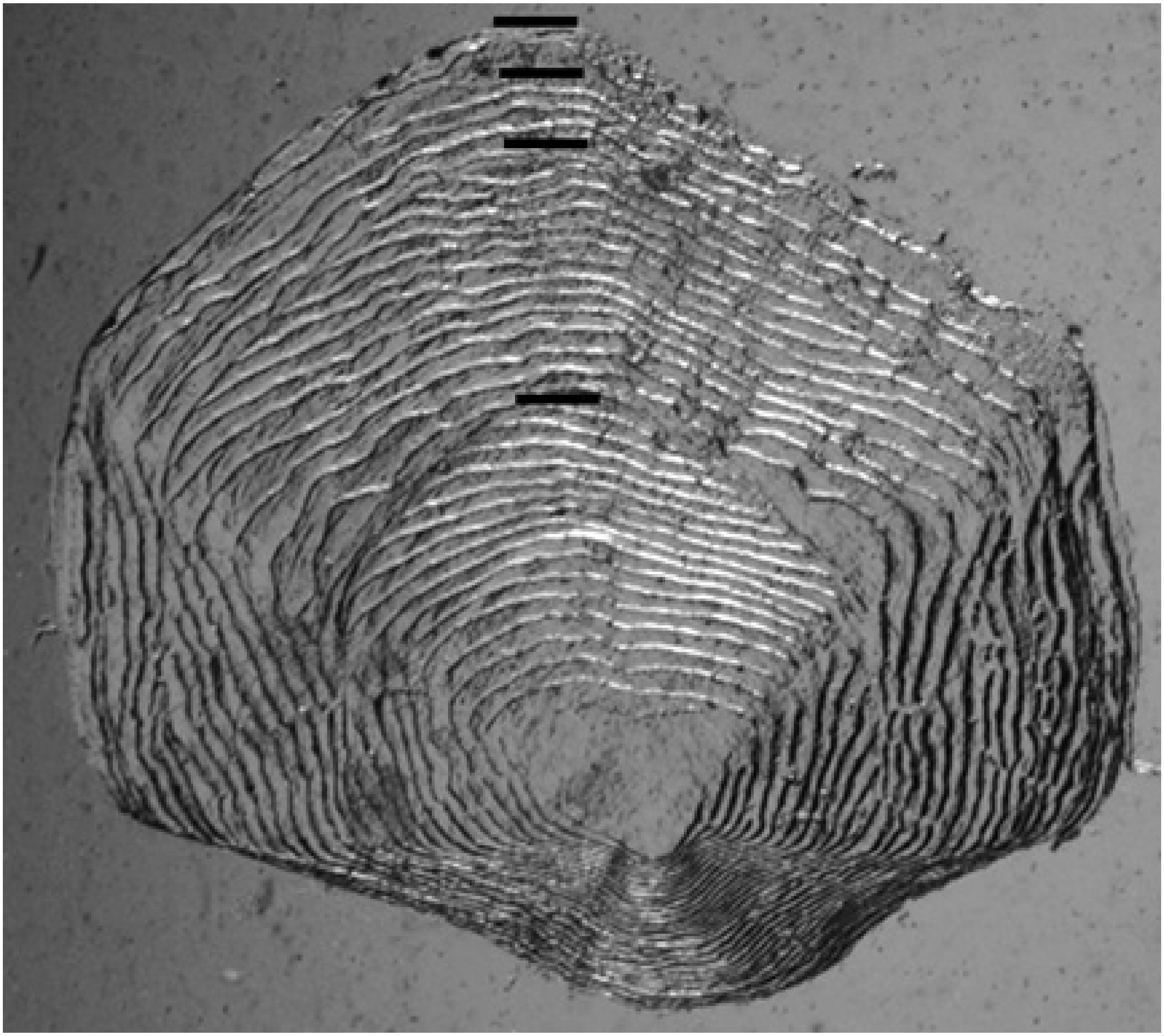


Figure 40. A scale from a 4-year-old rainbow smelt. Annuli are marked with bars. Note the little amount of growth between annuli 2, 3, and 4.

Striped Bass
Morone saxatilis

Striped bass scale samples are collected from the recreational fishery, the commercial fishery, and from the Massachusetts Division of Marine Fisheries tagging program. Otoliths are collected from a subset of the recreationally caught fish. Most fish aged range from two to twelve years old, although fish twenty years and older are not uncommon. Otoliths yield more precise ages, especially for older fish (>10 years), but are time consuming to remove and involve head mutilation, which is discouraged in the commercially captured fish.

Sample Collection

- Length, sex, capture date, location, and sample ID number are recorded on the sample envelope and data sheet.
- Otolith Extraction:
  - o Two methods can be used to remove the otoliths.
    1. The head is cut vertically, just behind the pre-opercula, using a reciprocating saw (Figures 41 and 42)
    2. The top of the head is removed just above the eyes using a reciprocating saw. A vide of this can be seen here: http://youtu.be/oC9tPCbOMAc.
  - o With an ideal cut, the otic capsules should be visible.
  - o Otoliths are removed using forceps.
  - o Otoliths are then rinsed, dried, and stored in labeled envelopes.
- Scale Collection:
  - o Scales are collected ventral of the first dorsal fin as shown in Figure 43.
  - o Mucus and any other debris are removed by scraping with a knife from anterior to posterior.
  - o 10-20 scales are collected with a clean knife and placed into corresponding envelope.

Scale Preparation and Ageing

- Preparation
  - o Three to five clean scales, with radii coming to a clear point at the focus, are selected (Figure 44).
  - o Regenerated scales are avoided (Figure 45).
  - o Scales are placed on a sheet of acetate, rough (distal) side down.
  - o Samples are pressed at 12,000 lb. for 3 minutes at 100°C in a heat press.
  - o Scales are removed from the acetate when cool.
  - o Each sample is labeled with corresponding sample ID number and put back into the corresponding envelope.
- Age Interpretation
  - o Scale impressions are viewed with transmitted light on image analysis software and a camera on a macro mirror stand.
  - o The mirror and lighting are adjusted so the annuli can be viewed cutting over near the baseline (Figure 46).
  - o Annuli are counted from the middle outward.
  - o Annuli appear as a disturbance in the circuli throughout the anterior portion of the scale and also cut over near the baseline (Figure 46).
  - o On older fish, annuli will become crowded near the edge of scales.
  - o Fish captured between January 1 and June 30 will have the edge counted as the last annulus.
  - o Fish captured between July 1 and December 31 will have growth beyond the last visible annulus but the edge is not counted.

Figure 41. Proper cutting axis on a striped bass.

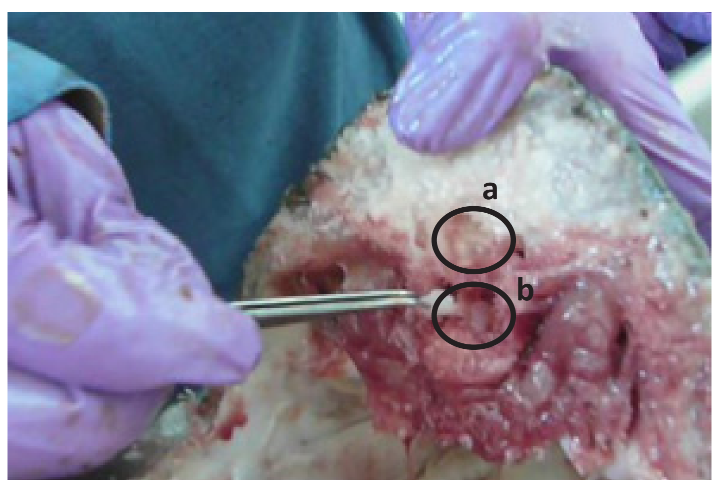


Figure 42. Location of otolith cavity (b) near the brain cavity (a) in a striped bass skull.

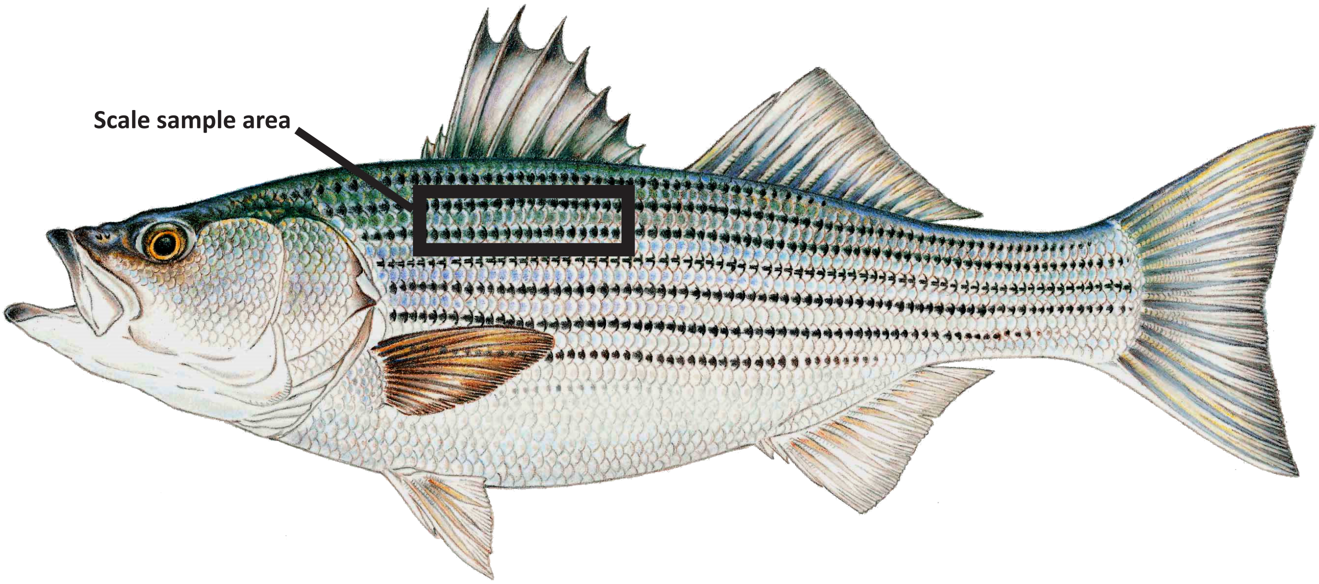


Figure 43. Sample area on a striped bass indicated by the rectangle.

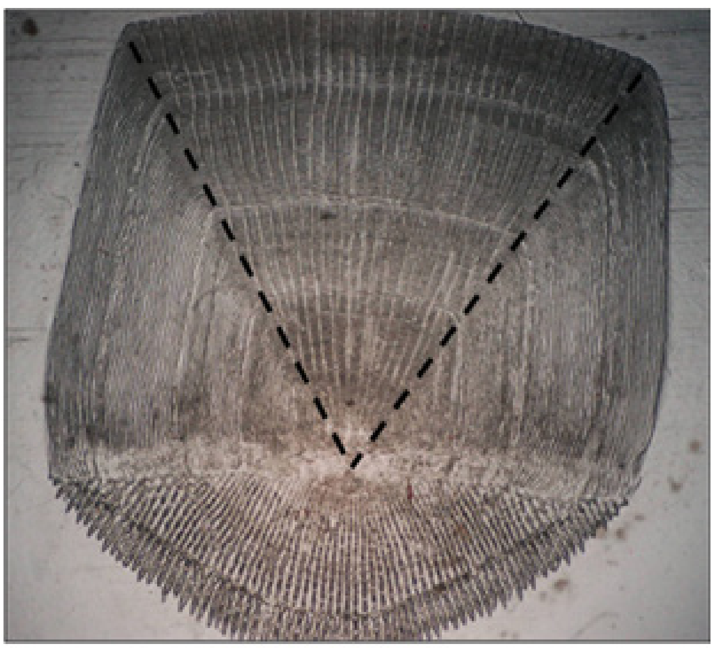


Figure 44. This scale is appropriate for ageing. Note how the radii form a V-shape. Image courtesy of Old Dominion University Center for Quantitative Fisheries Ecology, Virginia.

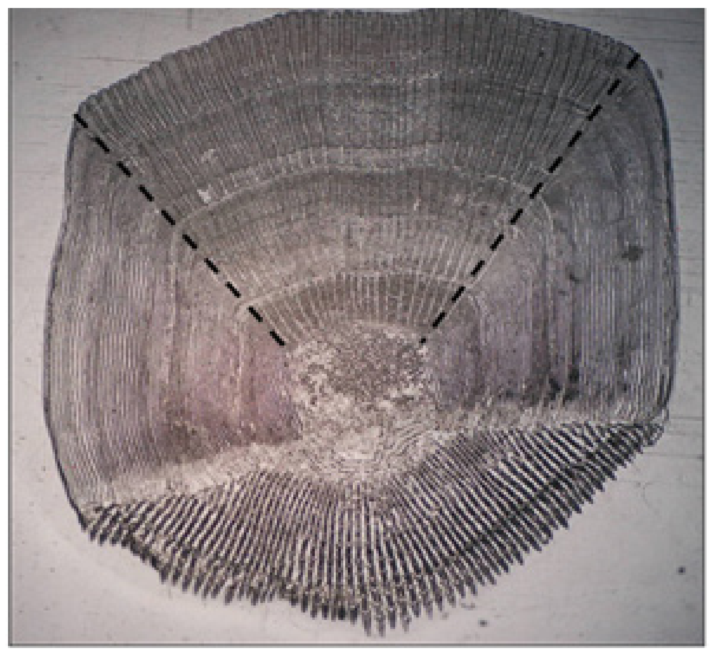


Figure 45. This scale has a regenerated center. Image courtesy of Old Dominion University Center for Quantitative Fisheries Ecology, Virginia.

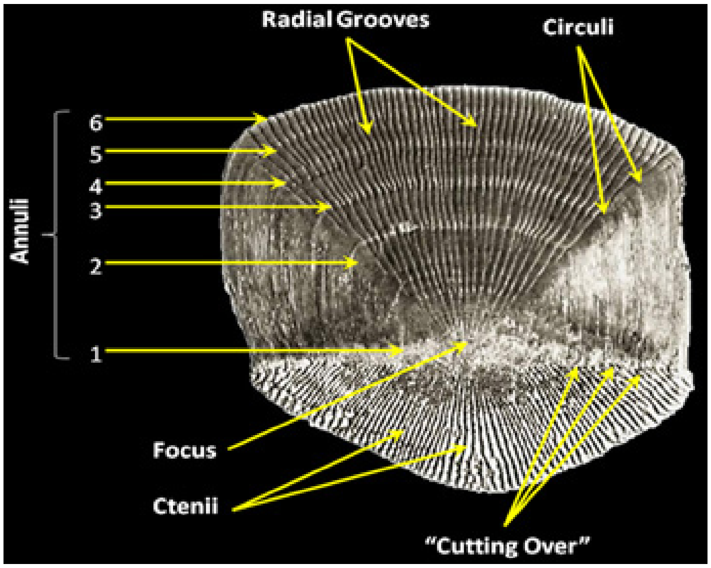


Figure 46. Scale from a 6-year-old striped bass. Image courtesy of Old Dominion University Center for Quantitative Fisheries Ecology, Virginia.

Otolith Preparation and Ageing

- Preparation
  - o Otoliths are mounted sulcus side up on glass slides with Crystal Bond.
  - o Using an IsoMet low speed saw with minimal weight, the otolith is cut through to create a transverse thin-section approximately 0.4 mm thick encompassing the core (Figure 47).
  - o The otolith section is placed in a labeled microcentrifuge tube.
  - o An empty porcelain well tray is heated in a 400ºC oven.
  - o After the tray is removed from oven, the otolith sections are placed into the wells to "bake."
  - o A properly heated tray will hold enough heat that the otoliths will "bake" quickly without needing to return the try to the oven.
  - o Otolith sections are removed from the tray when they turn golden brown.
  - o Each section is affixed to a pre-labeled slide with Flo-Texx liquid cover slip.
  - o Care is taken to remove any air bubbles that form in the Flo-Texx.
- Age Interpretation
  - o Otolith sections are viewed using transmitted light with a compound scope at 100x.

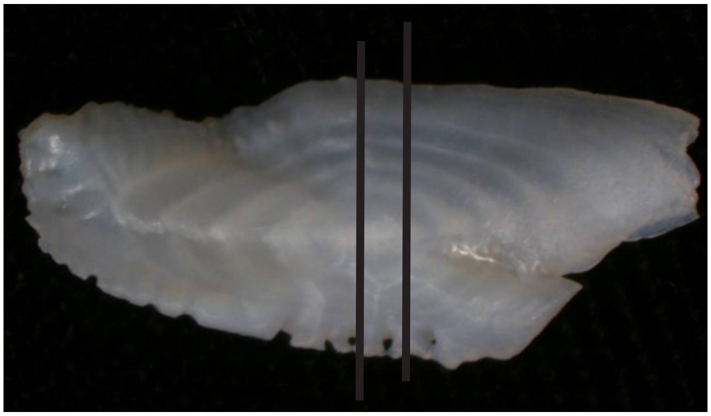


Figure 47. The appropriate axis for a transverse thin-section through the otolith core.

  - o Annuli are counted along the dorsal side of the sulcal groove (Figure 48).
  - o Care is taken to make sure all annuli counted are in the same plane of focus.
  - o A good section will have a V-shaped sulcus that extends to the core (Figure 48).
  - o A poor quality section will have a tornado shaped sulcus that might not extend to the core (Figure 49).
  - o Poorly cut sections may look better when the slide is flipped over.
  - o Grinding or polishing can be used to improve the readability of poorly cut sections.
  - o Fish captured between January 1 and June 30 will have the edge counted as the last annulus.
  - o Fish captured between July 1 and December 31 will have growth beyond the last visible annulus but the edge is not counted.

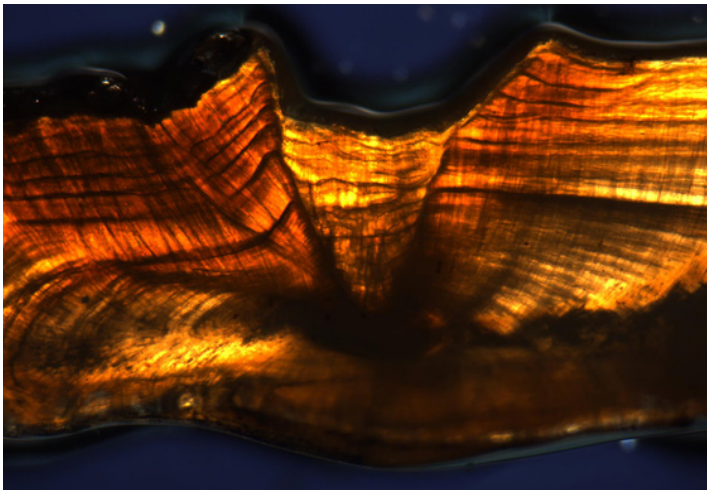


Figure 48. A good section from a a7-year-old striped bass captured in August. Note the sharp V-shaped sulcus.

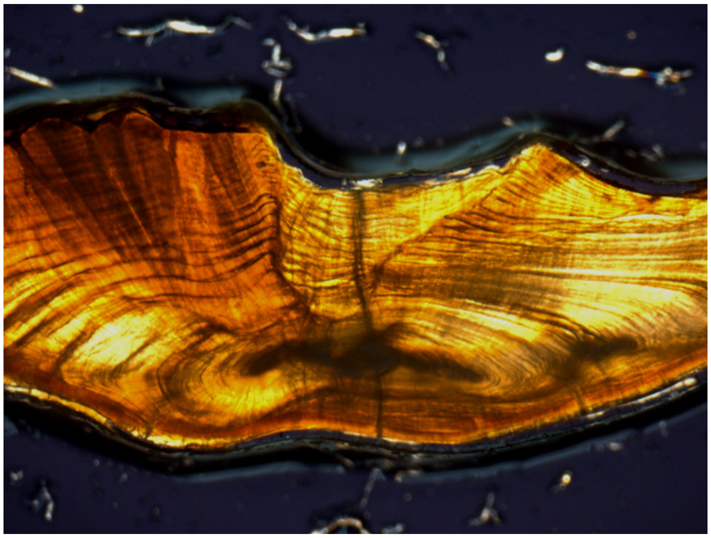


Figure 49. An example of a poor quality cut. Note the tornado-shaped sulcus.

Tautog
Tautoga onitis

Tautog are collected from both commercial and recreational fisheries. Opercula have been the primary structure used for ageing tautog, as they are easy to process and can be aged with the naked eye. The first annulus on opercula can become obscured in older fish, so otoliths have recently been added as an ageing structure. Otoliths can provide a clearer view of the first annulus and are very helpful when looked at in conjunction with the opercula.

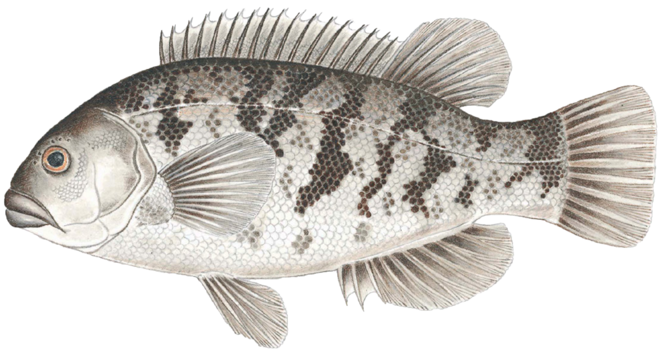


Figure 50. Tautog (*Tautoga onitis*)

Sample Collection

- Length, weight, sex, and location are recorded on waterproof Rite in the Rain® envelopes or plastic sample bags.
- Opercula are removed with a knife and placed in a labeled envelope. Care is taken not to cut through bone; a combination of cutting and tearing works best.
- Otoliths are extracted using a knife and forceps.
    - o The top part of the head is removed with a serrated knife or saw, exposing the brain cavity.
    - o Fine tip forceps are used to remove otoliths, which are located at the back of the brain cavity.
    - o Otoliths should be rinsed with water and stored dry in labeled micro centrifuge tubes.

Structure Processing

Opercula

- Opercula are submerged in boiling water for approximately two minutes.
- Upon removing from boiling water, any excess flesh is removed with a small brush.
- Opercula are rinsed in clean water allowed to air dry in labeled envelopes prior to ageing.

Otoliths

- Water is used to clean off any dried blood.
- One otolith is sectioned per fish.
- An empty porcelain well tray is heated in a 400°C oven.
- After the tray is removed from oven, the otoliths are placed into the wells to “bake.”
- A properly heated tray will hold enough heat that the otoliths will “bake” quickly without needing to return the try to the oven.
- Otoliths are removed from the tray when they turn golden brown.
- Otoliths are then embedded in two part epoxy in silicon bullet molds.
- Under a stereoscope, each block is marked with pencil through the core perpendicular to the sulcus.
- An IsoMet low speed saw is used to cut through the otolith core to create transverse thin-sections approximately 0.4 mm thick.
- The thin-sections are affixed with Flo-Texx liquid cover slip to slides labeled with their corresponding sample ID numbers.
- Care is taken to remove any air bubbles that form in the Flo-Texx.

Age Interpretation

Opercula

- Opercula are viewed with the naked eye using a combination of transmitted and reflected light.
- Annuli are counted from the opercula joint outward to the margin (Figure 51).
- The first annulus will frequently be hidden due to the thickness of the operculum.
- True annuli will be visible across the entire operculum (Figure 52).
- Fish collected in the spring will have an annulus forming at the margin (Figure 51)
- Fish collected in the fall will have growth after the most recent annulus (Figure 53).

Otoliths

- Otolith sections are viewed with transmitted light through a compound scope at 100x.
- Annuli are counted from the core outward.
- Care is taken to make sure all annuli counted are in the same plane of focus.
- Poor quality sections may look better from the opposite side.
- Grinding and polishing sections from older fish or poor quality cuts may facilitate easier ageing (Fgure 54).
- Fish collected in the spring will have an annulus forming at the margin, therefore the edge will be counted as the last annulus (Figure 55).
- Fish collected in the fall will have growth after the last annuli, therefore the edge will not be counted as the last annulus (Figure 56).

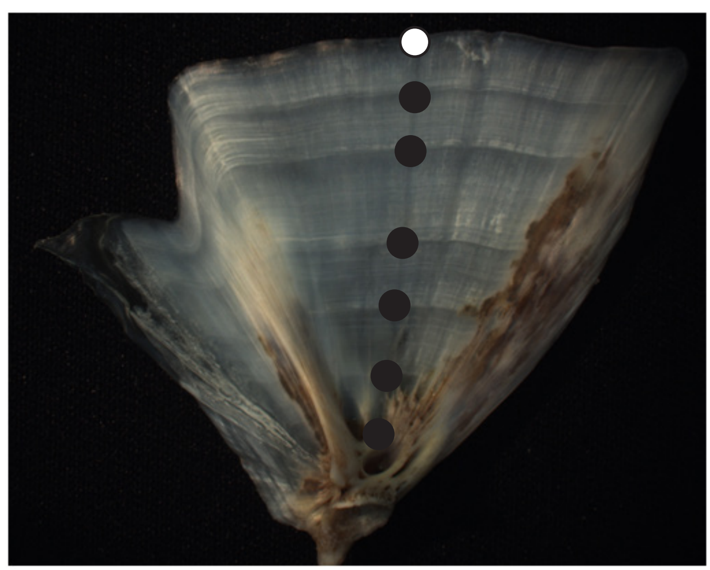


Figure 51. Operculum from a 7-year-old spring fish viewed under reflected light with annuli marked. The edge is counted as an annulus because this fish was captured in the spring.

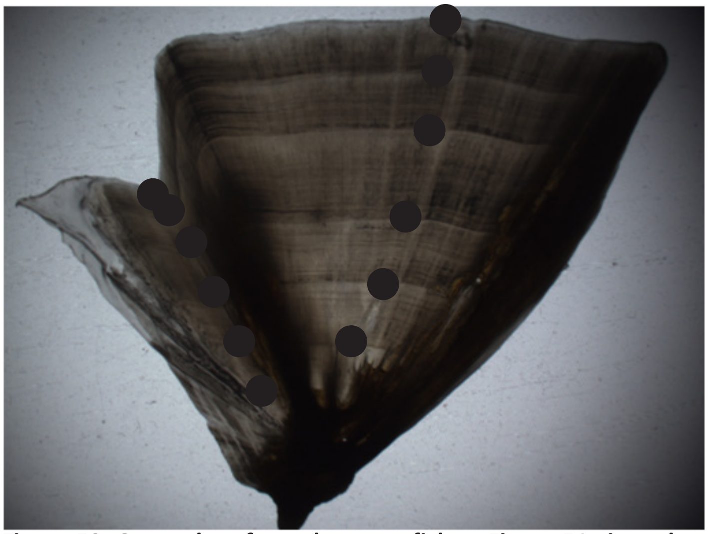


Figure 52. Operculum from the same fish as Figure 51 viewed under transmitted light. Note how the annuli can be followed across the entire structure.

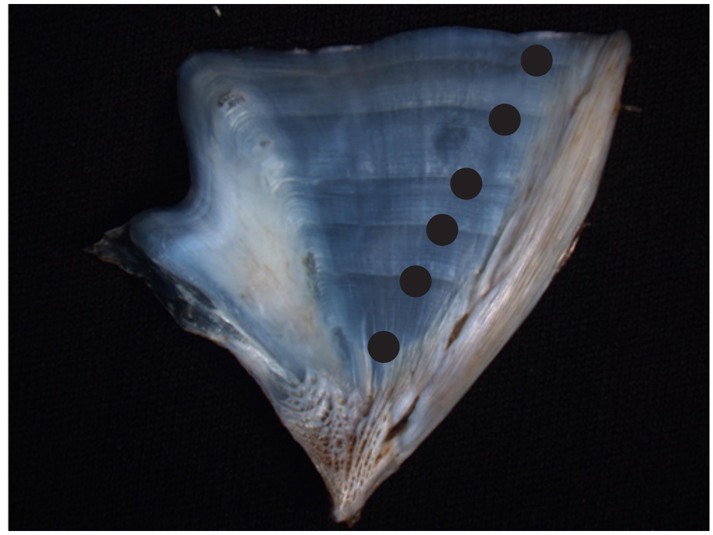


Figure 53. Operculum from a 6-year-old fish captured in the fall. The edge is not counted as an annulus.

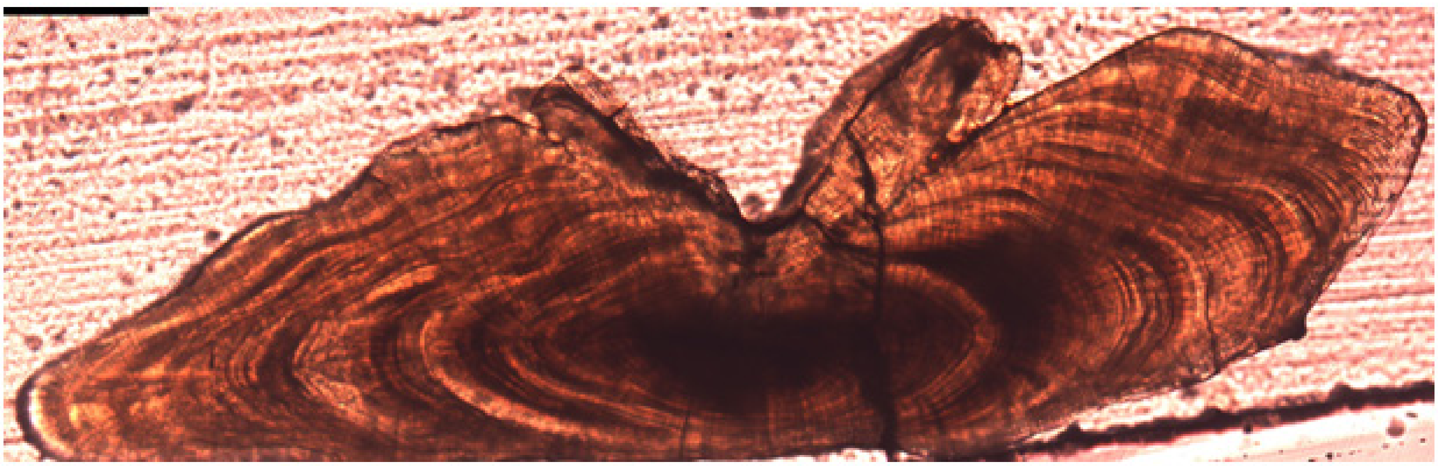


Figure 54. A ground down section of an otolith viewed under transmitted light. BEfore grinding, the annuli were very difficult to discern.

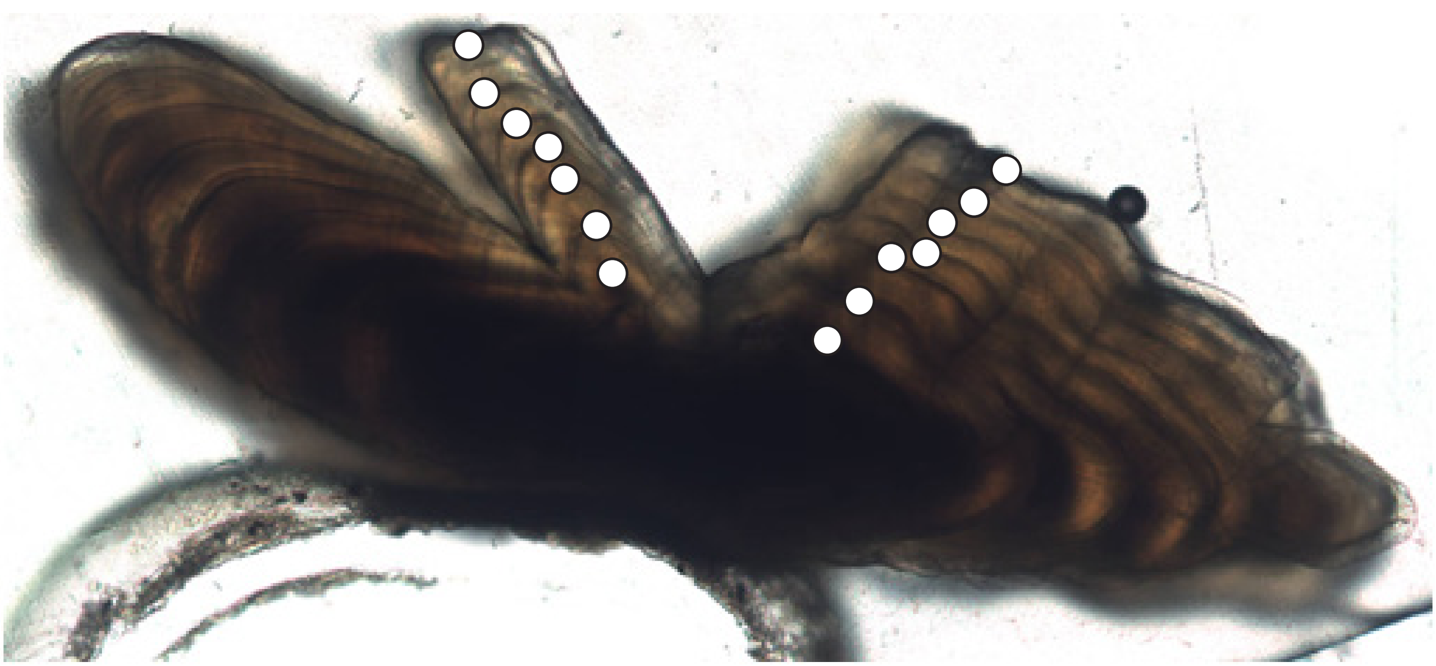


Figure 55. An otolith from a 7-year-old fish, captured in the spring, viewed under transmitted light.

Figure 56. An otolith from a 4-year-old fish, captured in the fall, viewed under transmitted light.

Winter Flounder
Pseudopleuronectes americanus

Winter flounder otoliths are collected through the Massachusetts Division of Marine Fisheries resource assessment trawl surveys in the spring and fall. Otoliths are initially read whole, however those aged five and older are then sectioned for more precise reading. Blind-side otoliths are preferred for whole ageing and eyed-side otoliths are preferred for sectioning.

Sample Collection

- Length, sex, capture date, location, and sample ID number are recorded on sample envelope.
- Otolith Extraction:
  - o A knife is used to make a cut into the head just anterior to the start of the opercula as seen in Figure 57.
  - o The head is folded in half and the otoliths are removed with fine forceps.
  - o Otoliths are stored dry in corresponding envelopes.

Whole Otolith Processing and Interpretation

- Water is used to clean off any dried blood.
- The blind-side otolith is preferred for whole ageing, however the eyed-side otolith may be used if the blind-side is unavailable. If both otoliths are available, it can be beneficial to view them together (Figure 58).
- Otoliths are immersed in mineral oil, sulcus down, on a black background.
- Otoliths are viewed under a stereomicroscope with reflected light.
- Annuli are counted outward from the core (Figure 59).
- Annuli are defined as continuous hyaline (dark) bands with no breaks (Figure 59).
- False annuli typically are not continuous, lack a defined edge, or split from true annuli (Figure 60).
- Fish collected in the spring will have an annulus forming at the margin, therefore the edge is counted as the last annulus (Figure 59).

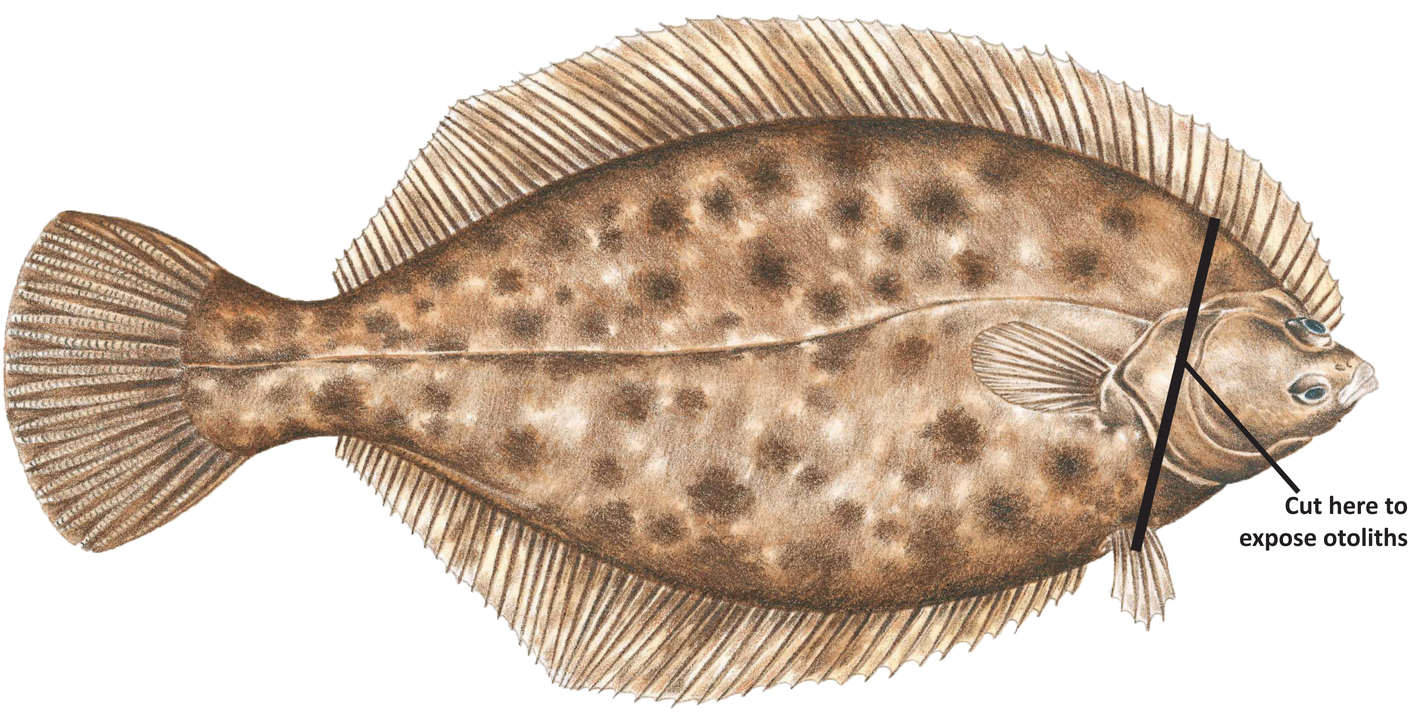


Figure 57. Incision area on a winter flounder.

• Fish collected in the fall will have growth after the last annulus, so the edge is not counted as the last annulus (Figure 60).
• Fish aged as five or older are sectioned to confirm age (Figure 61).
• An image of the whole otolith should be taken prior to sectioning.
• Otoliths should be stored dry to avoid clearing.

Otolith Section Processing

• Eyed-side otoliths are embedded in two-part epoxy (West Systems 105 Resin) in silicone bullet molds sulcus side up.
• The core of each otolith is marked on the epoxy block with pencil.
• A low speed IsoMet saw is used to cut through the otolith core to create transverse thin-sections approximately 0.4 mm thick (Figure 59).
• Otolith sections are stored in microcentrifuge tubes with corresponding sample ID numbers.

Otolith Section Interpretation

• Otolith sections are immersed in mineral oil on a black background.
• Sections are viewed under a stereomicroscope with reflected light. A compound scope using transmitted light at 100x can be used if age interpretation is difficult.
• Annuli are counted outward from the core along the sulcal groove (Figure 62).
• Annuli are defined as continuous hyaline bands with no breaks.
• False annuli are not continuous and often join with true annuli along the sulcal groove.
• Split 2nd annuli are seen frequently (Figure 62).
• The sulcus should be deeply "V" shaped with the point very close to the core (Figure 62).
• Sections with a shallow "U" shaped sulcus are not ideal and might be missing annuli (Figure 63).
• If needed, the whole otolith image can be referred to when determining the final age.
• Sections are stored dry to avoid clearing.

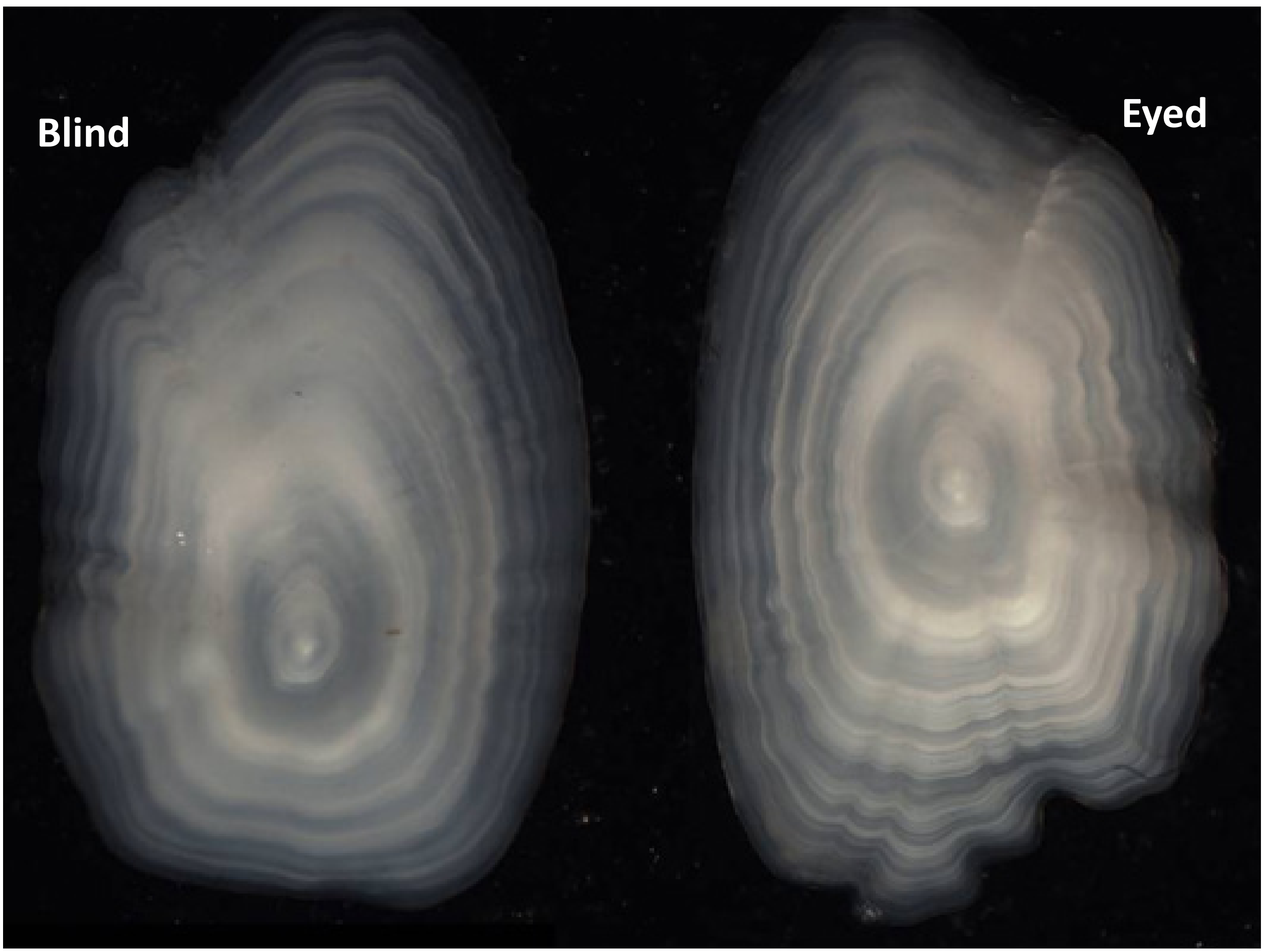


Figure 58. These are whole winter flounder otoliths from the same fish. Note the difference in the core position between the blind (left) and eyed (right) sides.

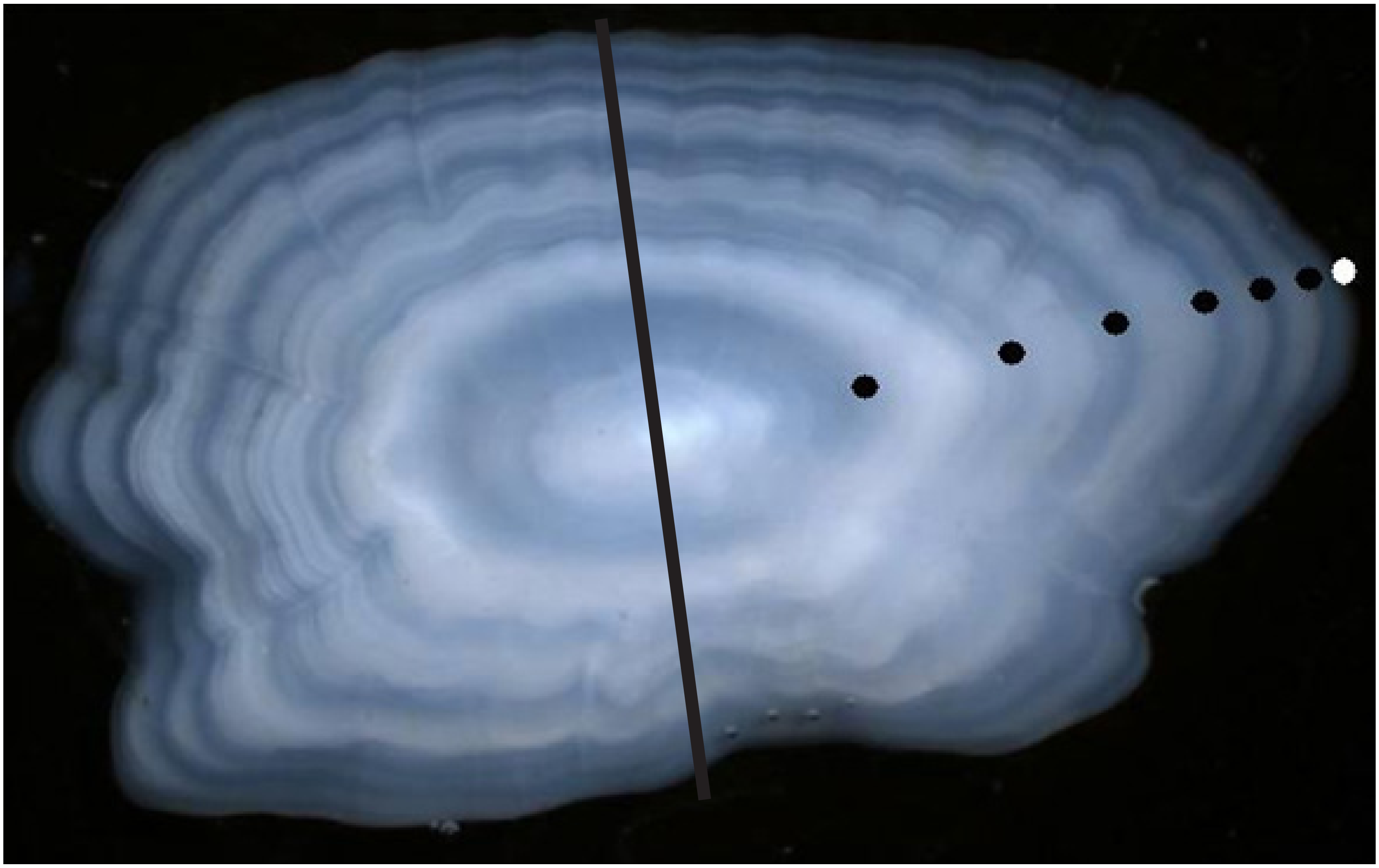


Figure 59. This is a 7-year-old otolith, with each annulus marked with a dot. This fish was captured in the spring, so the edge is counted as an annulus. A line marks the ideal sectioning axis.

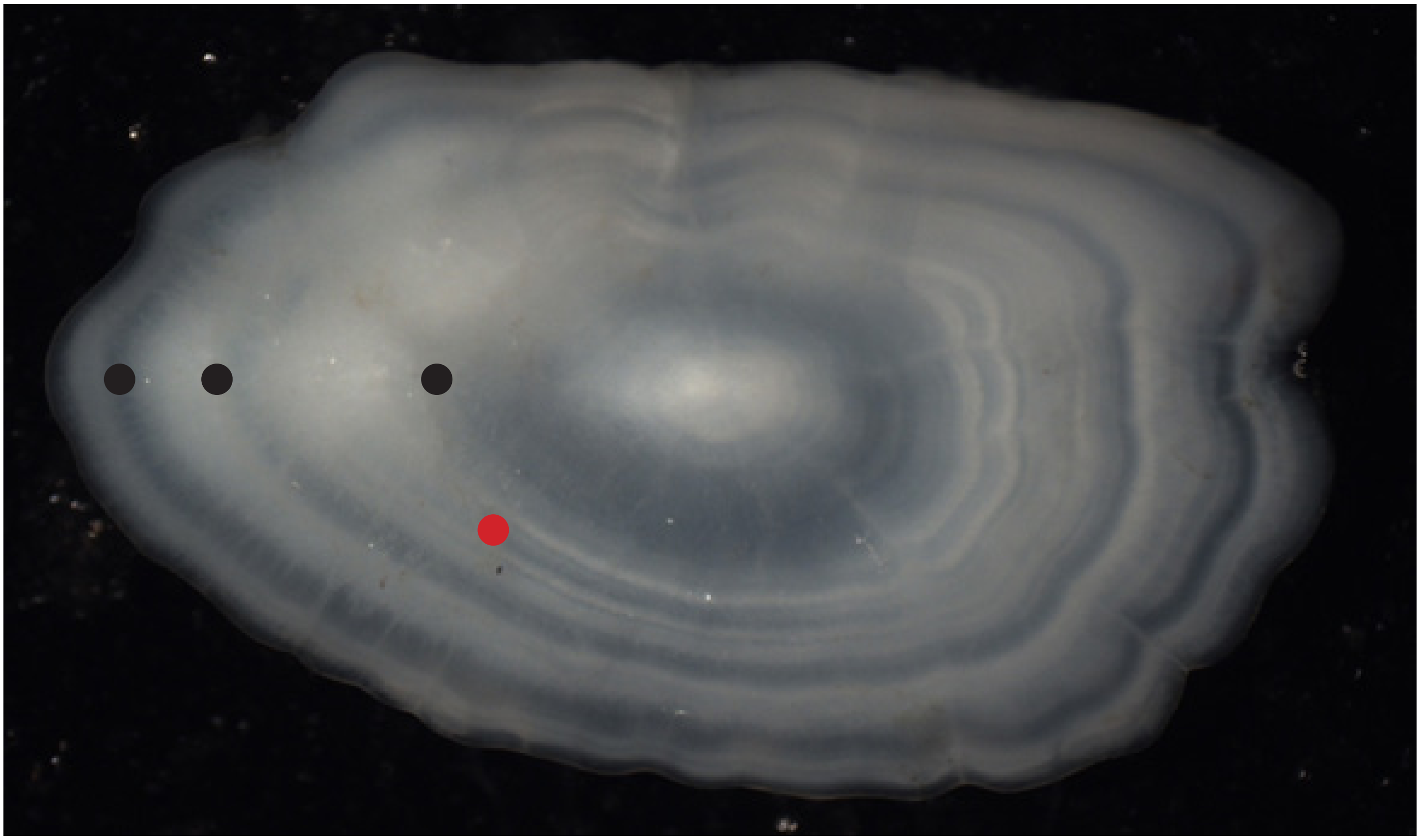


Figure 60. This is a 3-year-old, with each annulus marked with a black dot. The red dot marks a false annulus. Note how the false annulus is not continuous. This fish was captured in the fall, so the edge is not counted as an annulus.

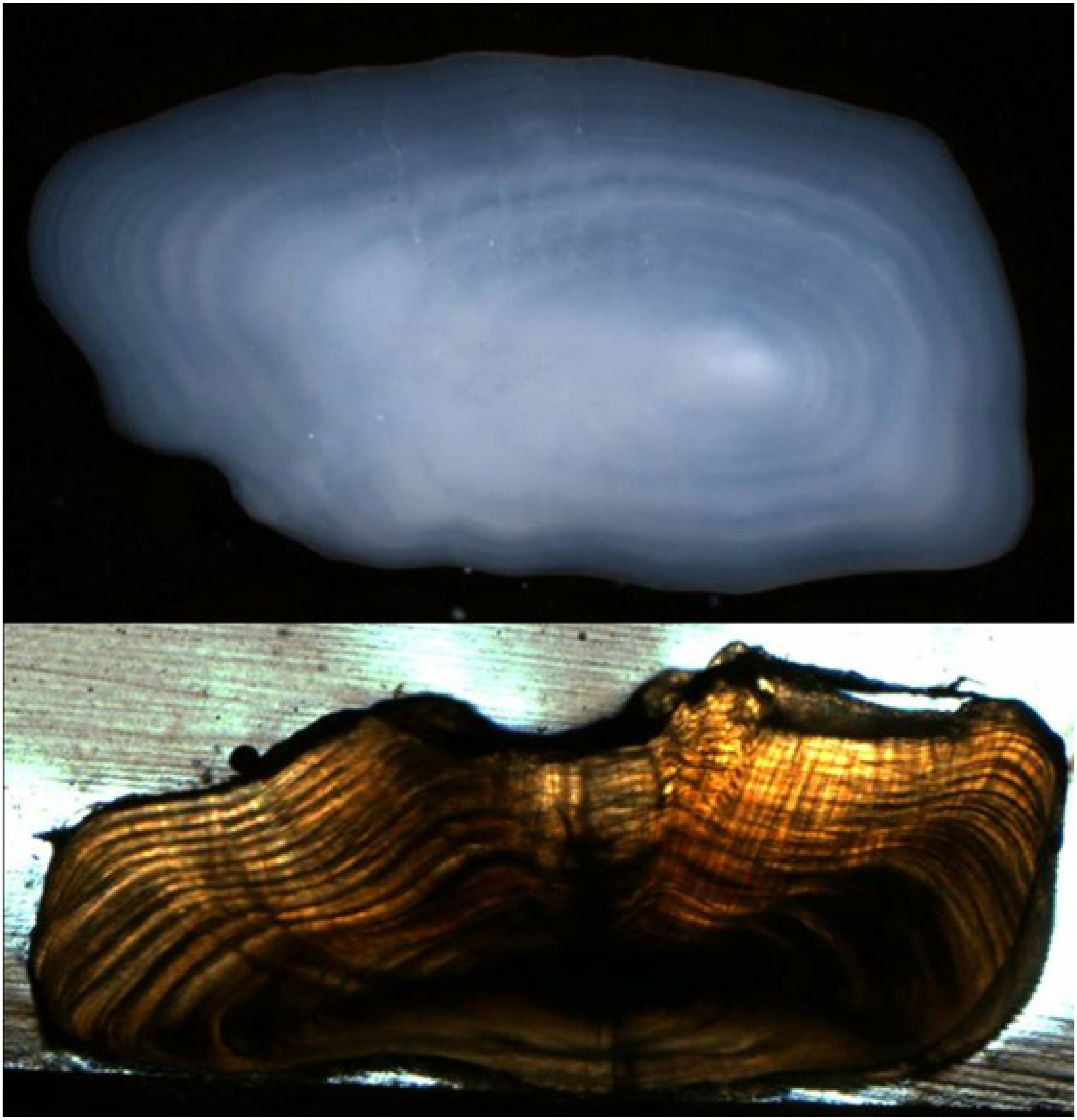


Figure 61. A whole otolith (top); the same otolith sectioned from a 13-year-old flounder (bottom). Note how the annuli stack near the outside edges of the section. Those stacked annuli are easily missed when the otolith is viewed whole. This is why sectioning is necessary on fish 5 years and older.

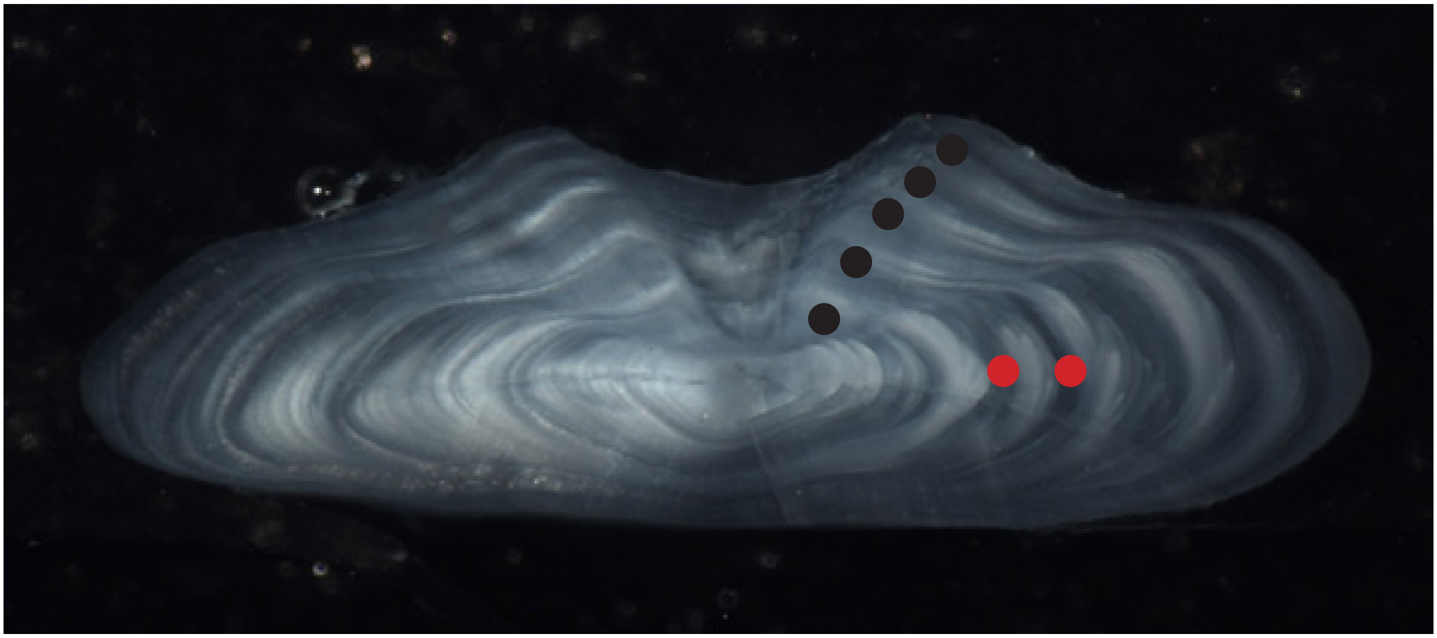


Figure 62. This is a well-cut otolith section from a 5-year-old flounder. Note that the sulcus is V-shaped and ends near the core. The annuli are marked with black dots along the ideal ageing axis. the edge is not counted as an annulus because this fish was captured in the fall. Note that the red dots are on marks that converge near the sulcus to become a single (2nd) annulus.

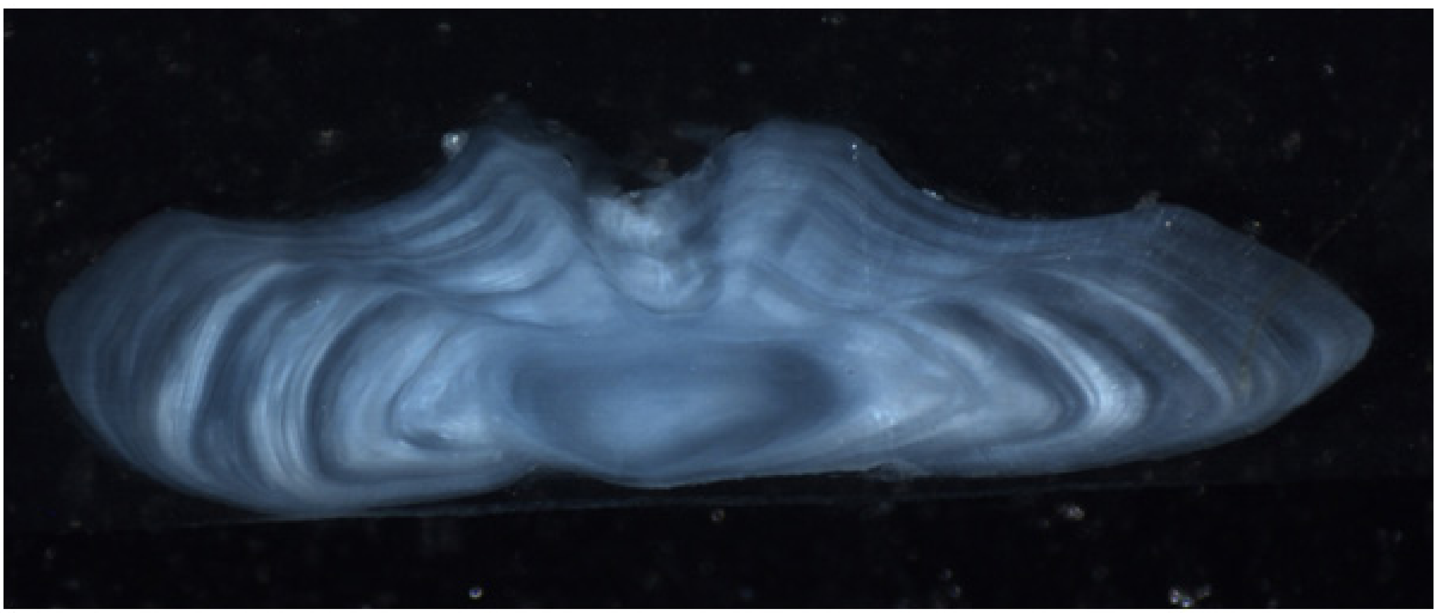


Figure 63. This is an example of a bad cut. Note how the sulcus is U-shaped and does not reach the core.

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