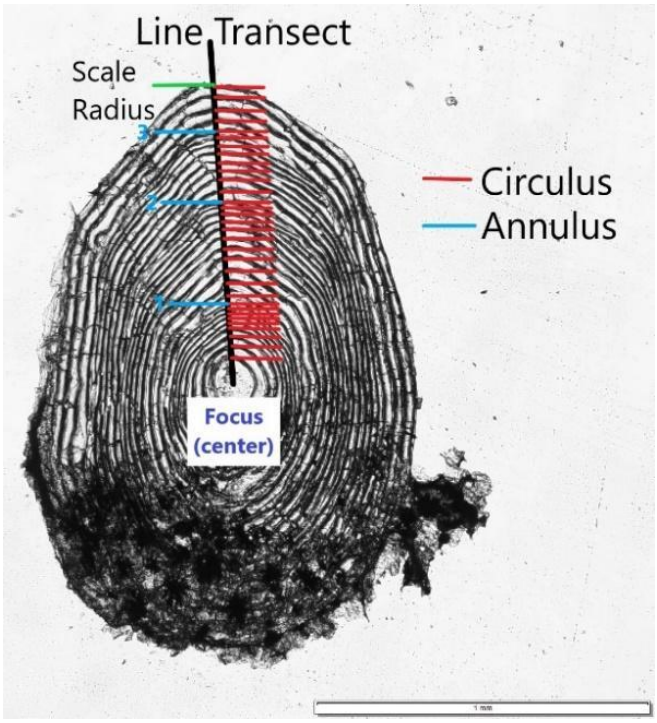
Northeast Fisheries Science Technical Memorandum 330

NOAA Fisheries Atlantic Salmon Ecosystems Branch Scale Image Analysis Protocols Version 2025.1



Northeast Fisheries Science Technical Memorandum 330

NOAA Fisheries
Atlantic Salmon Ecosystems Branch Scale Image Analysis Protocols
Version 2025.1

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US DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts

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Editorial Notes

Information Quality Act Compliance: In accordance with section 515 of Public Law 106-554, the Northeast Fisheries Science Center (NEFSC) completed both technical and policy reviews for this report. These pre-dissemination reviews are on file at the NEFSC Editorial Office.

Species Names: The NEFSC Editorial Office's policy on the use of species names in all technical communications is generally to follow the American Fisheries Society's lists of scientific and common names for fishes, mollusks, and decapod crustaceans and to follow the Society for Marine Mammalogy's guidance on scientific and common names for marine mammals. Exceptions to this policy occur when there are subsequent compelling revisions in the classifications of species, resulting in changes in the names of species.

Statistical Terms: The NEFSC Editorial Office's policy on the use of statistical terms in all technical communications is generally to follow the International Standards Organization's handbook of statistical methods.

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GLOSSARY

The definitions provided are based on Berg and Grimaldi (1967), Allan and Ritter (1977) and a 2011 International Council for the Exploration of the Sea (ICES) scale reading workshop.

Circulus – concentric line on the surface of the scale, comparable to a tree ring

Circuli – plural of circulus

Intercirculus spacing – the distance between two circuli on the scale; wide spacing indicates accelerated growth and narrow spacing indicates slow growth

Focus – the center of the concentric lines on the scale

Winter band – group of narrowly spaced circuli associated with reduced growth at low temperatures

Summer band – group of widely spaced circuli associated with accelerated growth at high temperatures

Summer Maximum – the circulus following the largest intercirculus spacing in the summer band

Winter Maximum – the circulus following the smallest intercirculus spacing in the winter band

Annulus – the end of the winter band, indicating the end of an annual zone on the scale

Annuli – plural of annulus

Growth check – narrow spaced circuli that are fewer in number when compared to winter or summer bands, comparable to a false annulus

River zone – the region on the scale that is associated with freshwater growth, extending from the focus through the last freshwater annulus

Sea zone – the region on the scale that is associated with marine growth, extending from the first marine circulus to the edge of the scale; it may contain freshwater growth in spawning adults

Plus-growth – region of widely spaced circuli that follows the last annulus on a scale

Run-out – growth occurring between the last freshwater annulus and the start of marine growth than can be difficult to identify due to gradual widening of circuli spacing

First Marine Circulus – the circulus that indicates the start of marine growth. Accuracy of identification can be affected by the presence on run-out on scale

Spawning mark – erosion of scale that occurs during the spawning migration

INTRODUCTION

This document provides a compilation of protocols used in the NOAA NEFSC Atlantic salmon Image Analysis labs. We describe detailed aspects of Atlantic salmon scale collection, mounting scales between microscope slides, imaging, aging, and measuring scale features. We have verified our procedures using CellSens Entry versions 3.2 and 4.2 and Image-Pro version 10 (Media Cybernetics 2020). Procedures may require modifications for use with other software versions. We have included procedures for using ImageJ because it is Open Source software and provides a no-cost option for measuring scales. We conducted a preliminary comparison of results using Image-Pro and ImageJ, and found the measurements to be comparable. However, we observed that the user interface and workflow of Image-Pro are more powerful than those of ImageJ. As a result, the efficiency of using Image-Pro may justify the expense, provided the necessary funds are available. *This document was last updated in March 2025 as version 2025.1*.

Overview

NOAA Fisheries' Atlantic Salmon Ecosystems Branch (AtSEB) collects, mounts, images, ages, and measures Atlantic salmon scales to inform population dynamics and to support research related to age, growth, and survival. Atlantic salmon scales contain valuable information that biologists use to determine age, rearing origin, growth rates, spawning status, and size at marine entry (DeVries and Frie 1996; Lund and Hansen 1991). Information gained from salmon scales relies on the assumption of an isometric relationship between scale and somatic growth, a relationship also influenced by life stage and genetics (Burton et al., 2024). Circulus deposition on scales varies depending on several factors (life stage, growth rate, and temperature), but typically a new circulus forms every 5 to 16 days (Thomas et al., 2019). The relationship between circulus deposition and time informs interpretation of growth rates; accelerated scale growth results in increased circulus spacing, while slowed scale growth results in decreased circulus spacing. We identify the winter band on scales as narrowly spaced circuli that reflect slower growth because of reduced or nonfeeding at lower temperatures. The completion of the winter band, following the summer band, indicates the completion of an entire year's growth. Therefore, we determine the location of the annulus as the last circulus of the winter band.

Sampling Programs and Research

Maine Atlantic salmon scale sample archives date back to the 1960s. Sampling efforts continue today targeting different life stages (fry, parr, smolt, adult returns) and using various sampling techniques (electrofishing, rotary screw traps, fish lifts, etc.). These ongoing sampling programs inform the effectiveness of recovery efforts including hatchery supplementation, habitat restoration, improved fish passage, and dam removals. In addition, biologists use scale archives to better understand the relationship between salmon growth and survival. A team of state and federal biologists annually summarizes Atlantic salmon population status and recovery updates. These annual reports of the U.S. Atlantic Salmon Assessment Committee are stored in the NOAA fisheries online repository.

The sampling process begins with the capture of fish, employing various methods tailored to different salmon life stages to ensure the safe release and survival of the sampled individuals. The Maine Department of Marine Resources (DMR) operates traps integrated into dams to capture adult salmon during their spawning migration. In collaboration with AtSEB, DMR also captures

salmon smolts using rotary screw traps, which researchers deploy in select rivers during the smolts' annual spring migration to the sea. For sampling salmon parr, DMR captures fish by electrofishing, which temporarily stuns the fish. During each of these sampling processes, field researchers gather biological data, as well as scales and tissue samples, from the specimens. The scale samples are then sent to the laboratory where they are prepared for aging, imaging, and analysis. Age readers typically determine salmon ages during the process of taking high-resolution images of the scales. In cases where researchers have requested growth analyses, scale readers extract measurements from key scale features. To ensure accuracy, at least one additional scale reader conducts a data audit, reviewing both age determinations and scale measurements.

SCALE SAMPLING PROCEDURES

Scale Collection

Required Equipment:

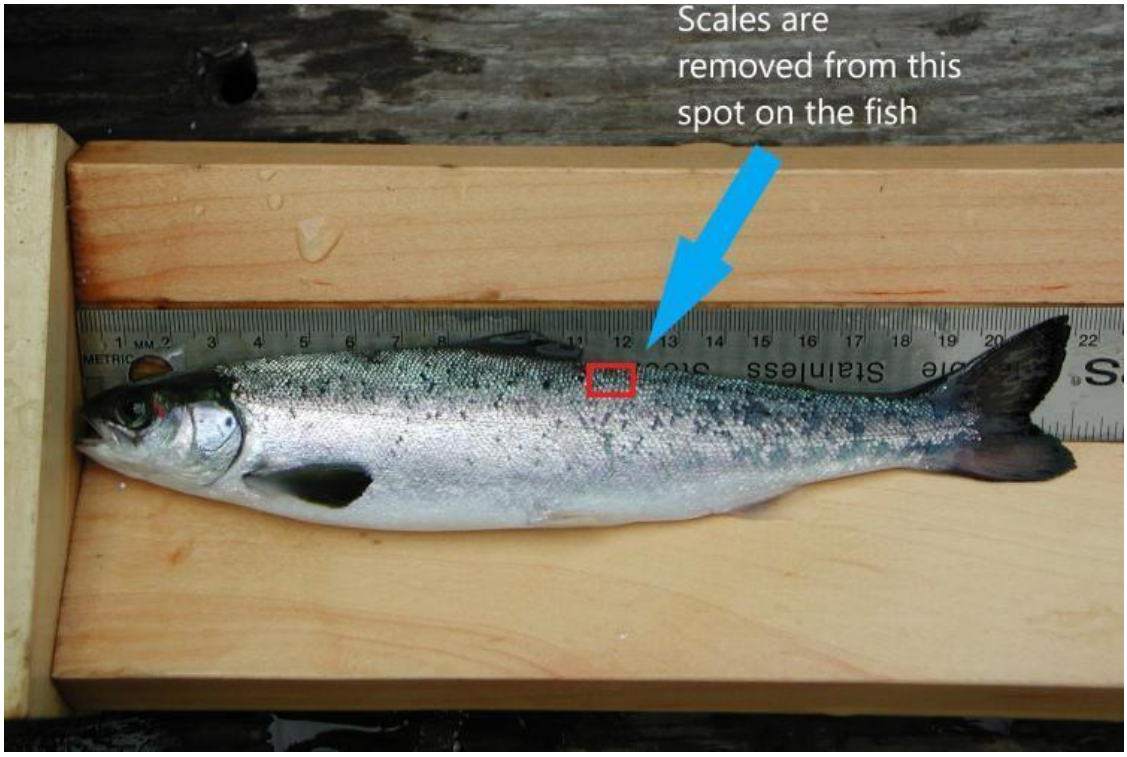
- Scale knife
- Scale envelopes with paper inserts
- Measuring board

Sampling:

1. Field samplers collect scales from Atlantic salmon based on ICES methods (1992)
    a. The location on salmon for scale collection is three to six rows above the lateral line on a line extending from the anterior edge of the anal fin to the posterior edge of the dorsal fin (see images on page 4).
    b. The sampler removes excess mucus from the region of interest on a salmon by scraping the back (dull side) of a knife from the head toward the tail.
    c. The sampler removes 10 to 20 scales by applying gentle pressure with the knife and making short, even swipes working from the anterior to the posterior end of the fish.
2. Field sampler places the knife with scales into a folded paper that is inserted into a coin envelope and squeezes the envelope while slowly pulling the knife out to transfer all scales from the knife to the paper.
    a. The sampler swipes the knife across the paper to spread the scales and minimize bunching and sticking together.
    b. The sampler air dries the envelopes slowly, away from direct light, before being stored.

Tips for Field Sampler:

1. Minimize stress on the fish by performing the following:
    a. Use a gentle but firm grip, covering most of the smolt in the hand.
    b. Complete scale sampling in under 30 seconds.
    c. Verify proper anesthetization before sampling.
    d. If no anesthetic is used, use two people for sampling—one holds, one samples.
2. Be sure to wipe down the scale knife regularly and double check that no scales remain from the previous fish (cross contamination of scale samples renders samples unusable).



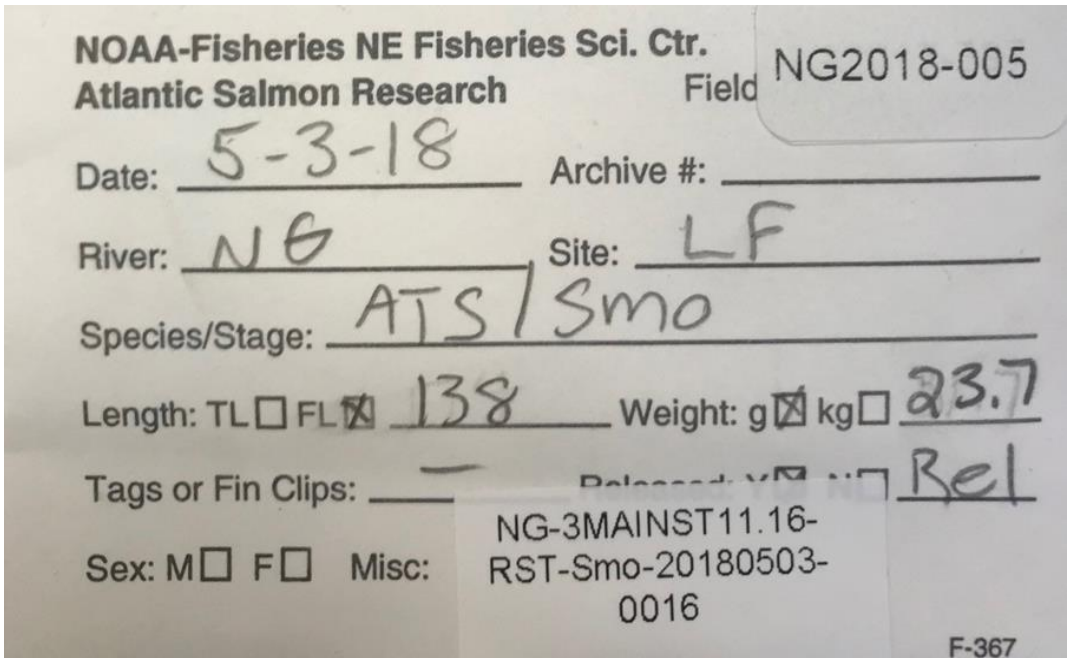
The smolt above has been sedated for approximately one minute to allow for handling without stress. ***Credit:*** *NOAA Fisheries/Ruth Haas-Castro*

An adult salmon is scale-sampled before being sold at a fish market in West Greenland. ***Credit:*** *NOAA Fisheries/Ruth Haas-Castro*

Scale Archiving

The NEFSC Atlantic salmon biologists store scales in envelopes with the following information;

- Unique Identifier: Fish ID code and/or JoinID (our term for this unique identifier)
- Sampling date
- Location
- Species
- Life stage
- Fork length
- Weight
- Clip/tag/mark information
- Notes



Example of a scale envelope (2x3 inches) for the Atlantic Salmon Ecosystems Branch. ***Credit:*** *NOAA Fisheries/Ruth Haas-Castro*

Physical Archiving

Because of the presence of historic scale samples with deteriorated labels, new standards on the quality of label are recommended. DO NOT USE REGULAR PRINTING MATERIALS that may fade or deteriorate over time.

Materials

- Archive grade labels
- Archive grade label makers
- Ink or printing ribbons
- Scale envelopes
- Box for scale envelopes
- Optional: Airtight containers or totes for boxes of scales

Important Information

1. Label all physical envelopes with both a unique identifier (JoinID) and a Field ID. Ensure both labels are visible and not stacked one over the other.
    a. We term this unique label a JoinID; this code contains information related to the river sampled, location of sampling (river km), sampling type, sampling date, life stage, and fish number.
    b. Field ID contains information related to river sampled, year, and fish number.

2. Store physical envelopes in appropriate scale boxes (sized to fit width and height of envelopes with little).
    a. Each day's samples should be separated by binding groups of envelopes with rubber bands.
        i. Avoid banding envelopes too tightly, as this can damage the envelopes and break glass slides.

3. Scale boxes should be labeled with river, year, and box number (e.g., #3 of 5) and placed either in a climate-controlled environment or in airtight totes.

For information on best practices for archiving scales and data, please view the ICES Scientific Report, ICES Workshop on Scale, Otolith Biochronology Archives (WKBIOARC).

SCALE MOUNTING PROCEDURES

Materials

Materials required for scale mounting (see list below for details)

- Two pairs of forceps
- Microscope slides
- Laboratory wipes or lens paper for cleaning slides
- Invisible write-on tape
- Weighted tape dispenser
- JoinID labels
- Extra fine point permanent marker for writing on slides
- Optional: One prong
- Optional: Stereo microscope

Scale Mounting Procedures

1. Clean slides if needed before placing scales on them. Dirt trapped between slides cannot be cleaned post-mounting without separating the slides.

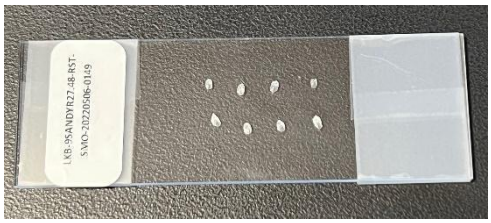
2. Remove 10 to 20 scales from the envelope—or as many as possible if there are not at least 10 available. Use a sharp pair of forceps for getting under scales stuck to paper and a second pair that can be blunt for gently pulling or teasing apart two or more scales that are stuck together or to the paper. *Be gentle*; avoid ripping scales and scratching the surface of scales. If necessary, use water to separate them. Scales with large amounts of debris can be cleaned by gently rubbing wet scales between two paper towels or using a soft-bristled brush. Ultrasonic cleaners can also be used, but use caution given the potential to cross contaminate scales.

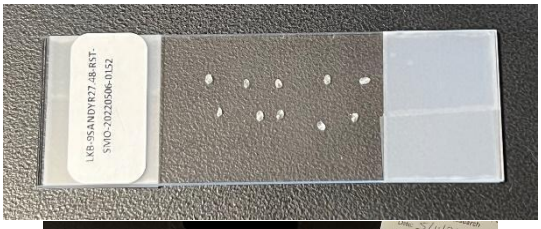
3. Align scales in straight rows on the slide—all oriented in the same manner, preferably with the posterior edges up. The posterior edge of the scale is the edge that sticks out of the fish and sometimes has pigmented flesh stuck to it. Mount the scales on the slide in the middle region, allowing margins on the ends for taping the slides together. Once the scales are arranged on the slide, cover the slide with another slide, securing the scales in between. Squeeze the slides together tightly as you wrap tape around each end (see photos on page 9). If tape is loose, scales may fall out. Place JoinID stickers on the slide's left end and on the associated scale envelope. The placement of JoinID labels depends on location of Field.

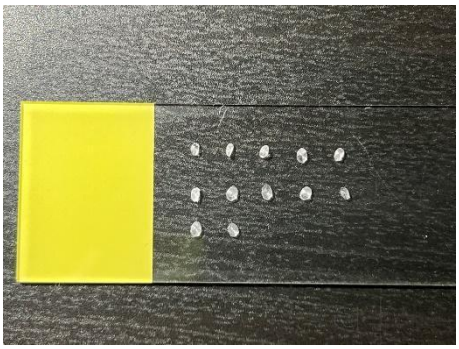
4. If a dissecting scope is available, use it to ensure the presence of some readable scales, that not all are regenerated or damaged. If all are regenerated, remove additional scales from the envelope and mount them on separate slide. Place **both** slides back into the scale envelope. Place the JoinID label on the slide with readable scales and use a permanent marker (archival quality) to label the additional slide with the JoinID and the "regenerated." The presence of regenerated scales and location of the regenerated material may be important information. If all scales are regenerated beyond readability, write "all regenerated" on the scale envelope.

Mounting Issues and Tips

Wrap tape tightly around the slides. If loosely wrapped, the scales may fall out. Squeeze the two slides together while wrapping the tape around the ends of the slides (see image **Well-Mounted Slides** on page 9).

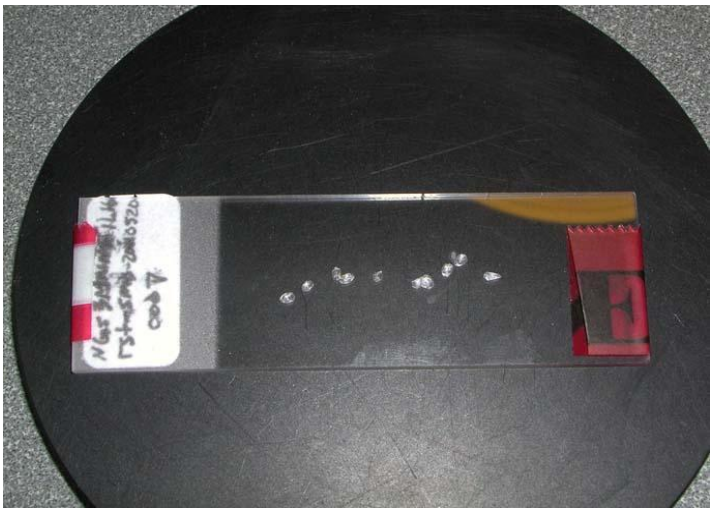


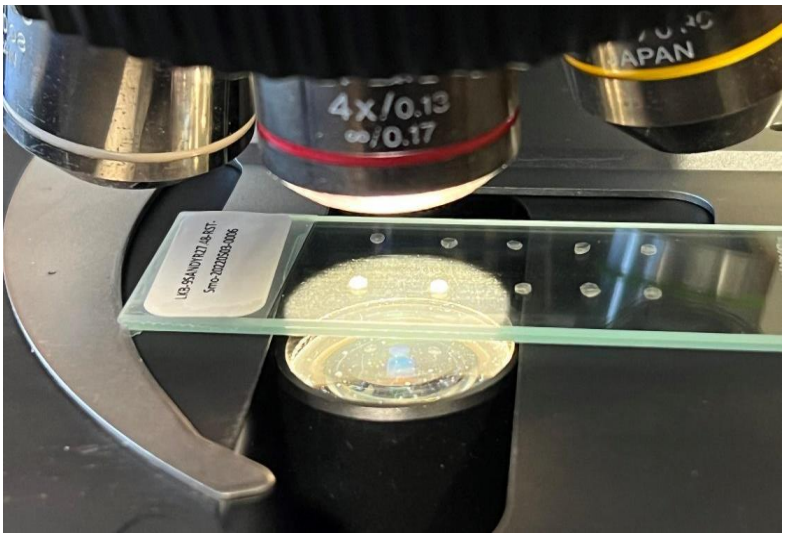




Well-Mounted Slides

Poorly Mounted Scales





Select readable scales, i.e., undamaged, folded, and piled on top of each other non-regenerated. A few regenerated scales are acceptable, but ensure most of the scales on the slide are non-regenerated.

Folded Scale

Regenerated Scale

Piled Scales

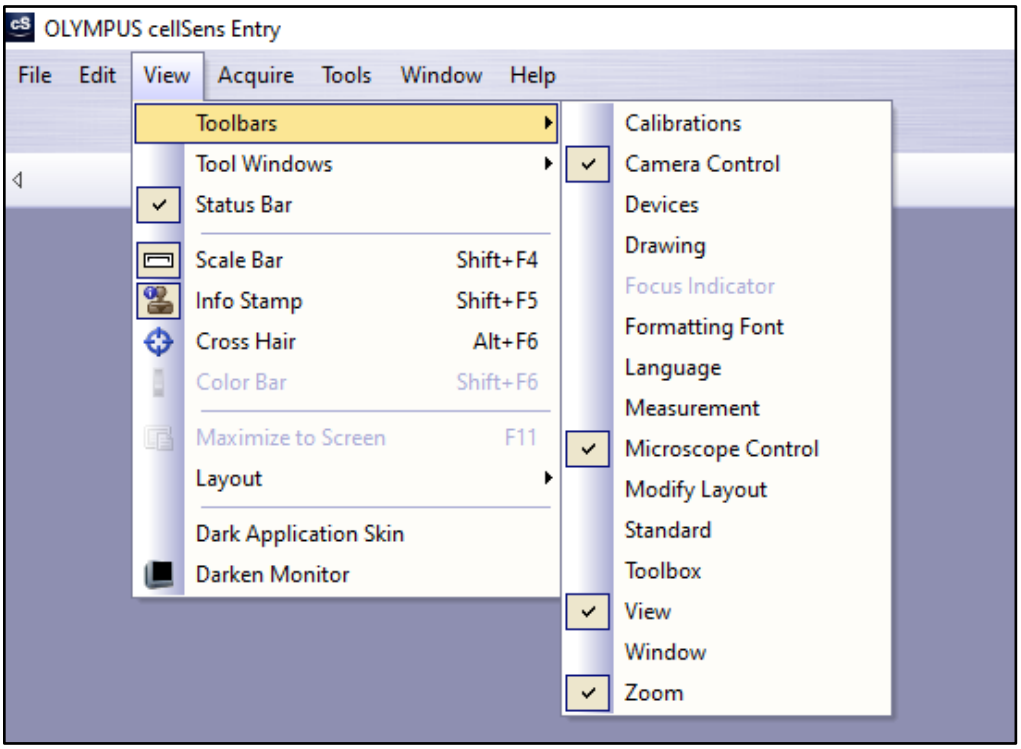
SCALE IMAGING PROCEDURES

CellSens (Olympus image capture software) Setup

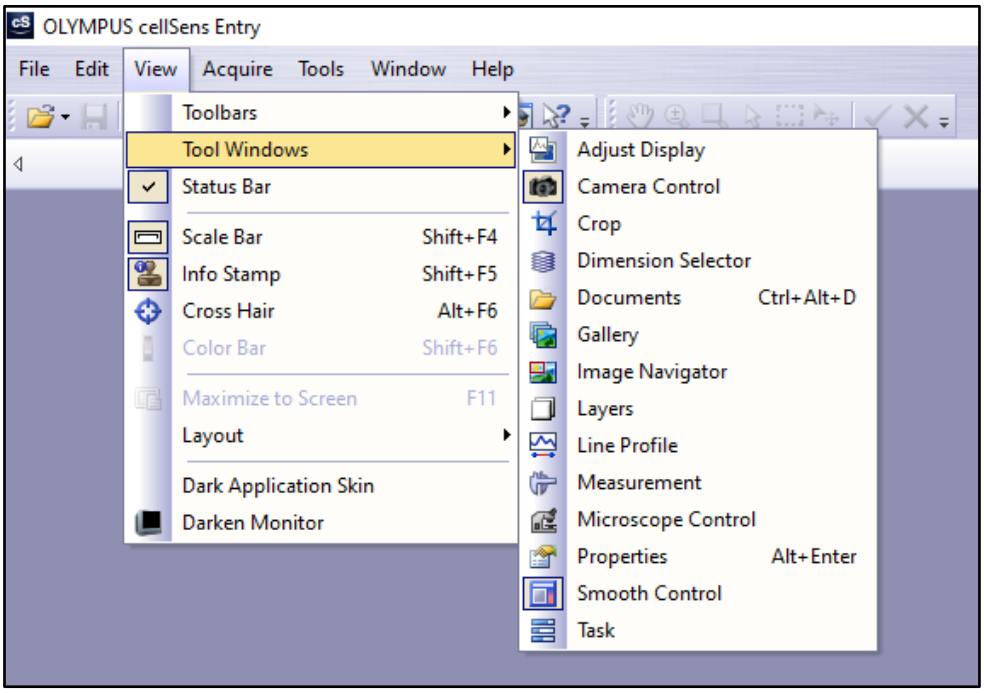
Procedures have been verified using CellSens Entry 3.2 and 4.2 (Olympus Corporation 2021). Other versions of CellSens Entry may need adaptations to procedures.

We recommend that the new user start by using the Help menu, which provides an overview of the user interface with information on how to adapt it to the user's needs. CellSens opens initially in the default layout named *Simple*, indicated by the tab in the top-right corner of the window under the **Layout** tab. Once changes are made to the layout, the new layout should be saved in a new tab with a meaningful name (e.g., "SmoltScales" or "AdultSalmonScales").

1. Set up the new layout for imaging salmon scales, start with **View** tab in the menu in the top-left of the window.
    a. Click **View** > **Toolbars** > **Camera Control, Microscope Control, View**, and **Zoom**



    b. Click **View** > **Tool Windows** > **Camera Control** and **Smooth Control**
    c. Click **View** > **Status Bar, Scale Bar,** and **Info Stamp**



2. Set up the **Camera Control** Tool Window.

   a. Move cursor to the toolbar at the top of **Camera Control** window

      i. *Grayscale Mode* (located at top of window) – toggle on by clicking 

      ii. (Optional) *Focus Indicator* – toggle on by clicking 

   b. *Exposure* – select *Automatic* for adjusting exposure

      i. Select size (or *region*) for Spot: 1% works well for most scales (only available in *Automatic* Exposure)

      ii. *Sensitivity*: 100

   c. *Resolution* – choose highest for both *Live* and *Snapshot*

      i. May choose lower resolution for smolt scales if storage space is limited

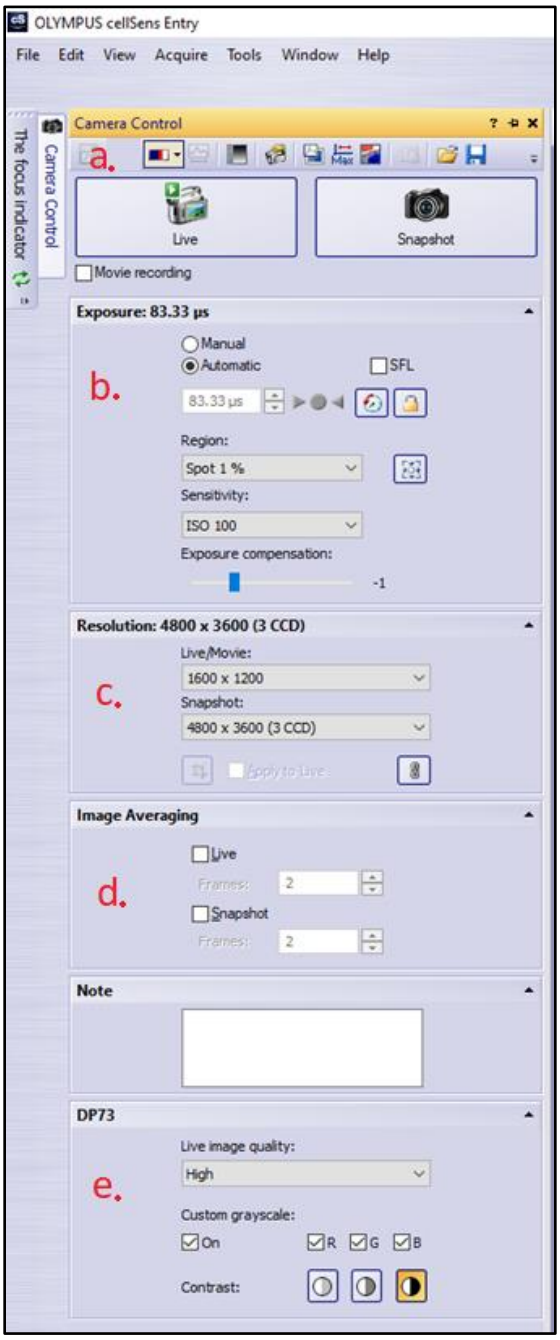
   d. *Image Averaging* – leave as default (set at 2 for both Live and Snapshot)

   e. Window with your camera name (DP73, DP74, etc.)

i. *Live image quality*: select **High**

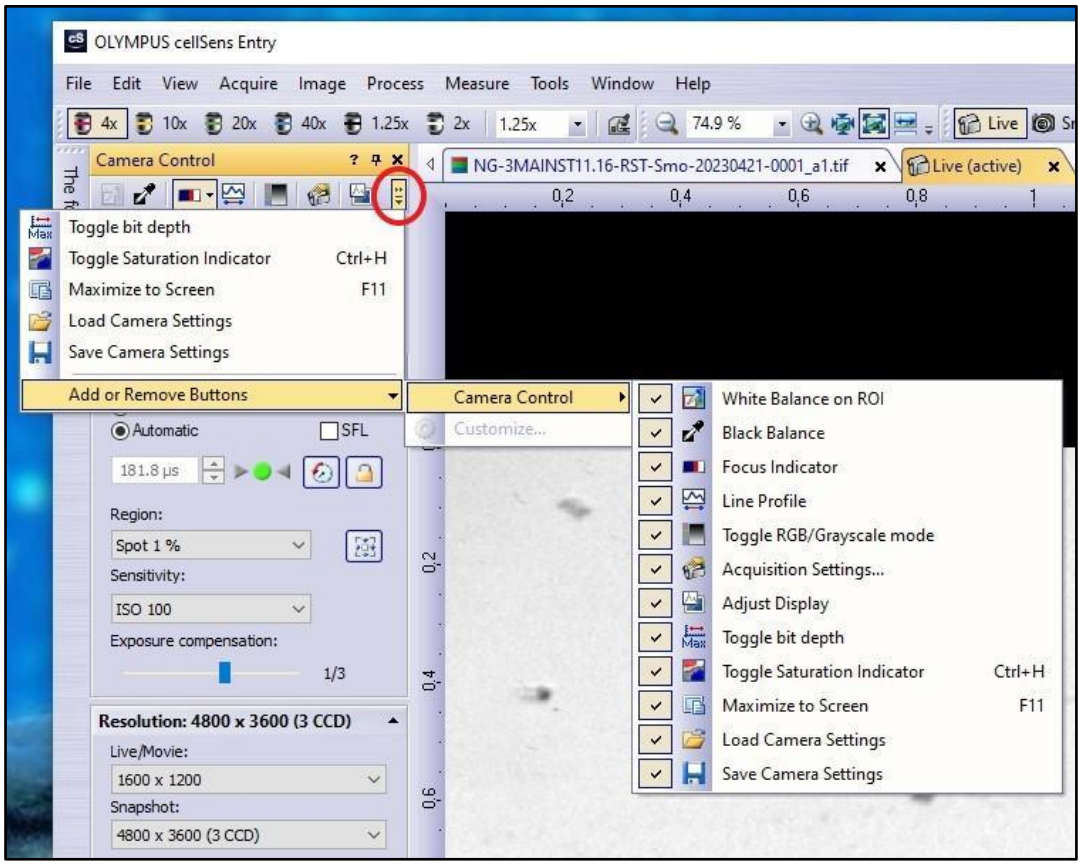
ii. *Custom grayscale*: check **On, R, G, B**

iii. *Contrast*: High

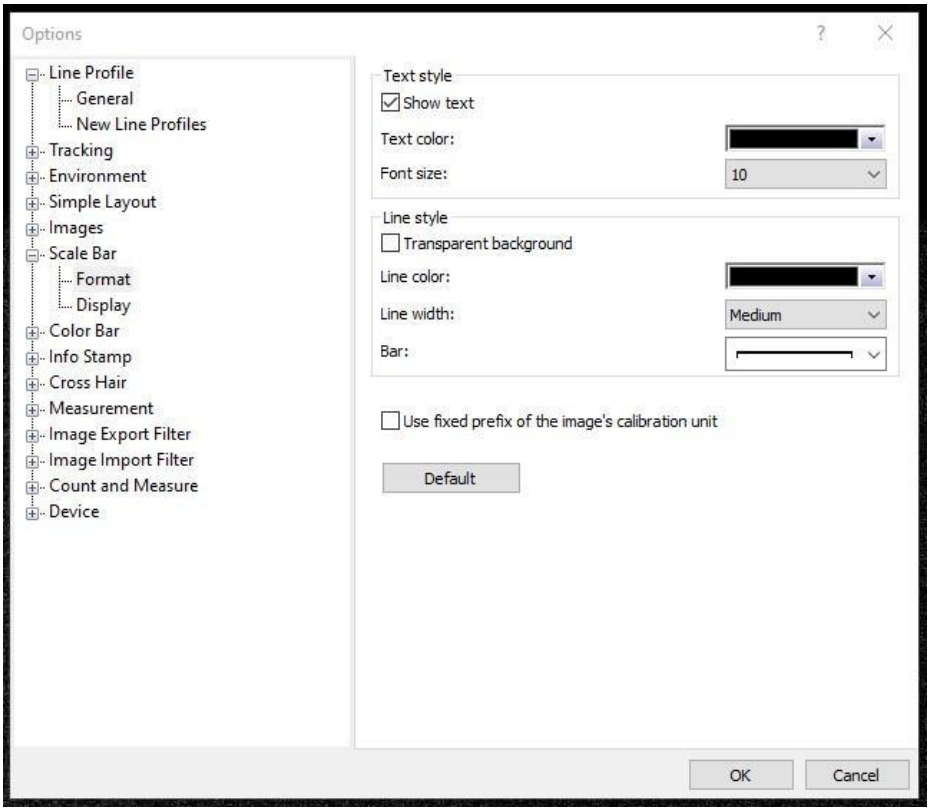


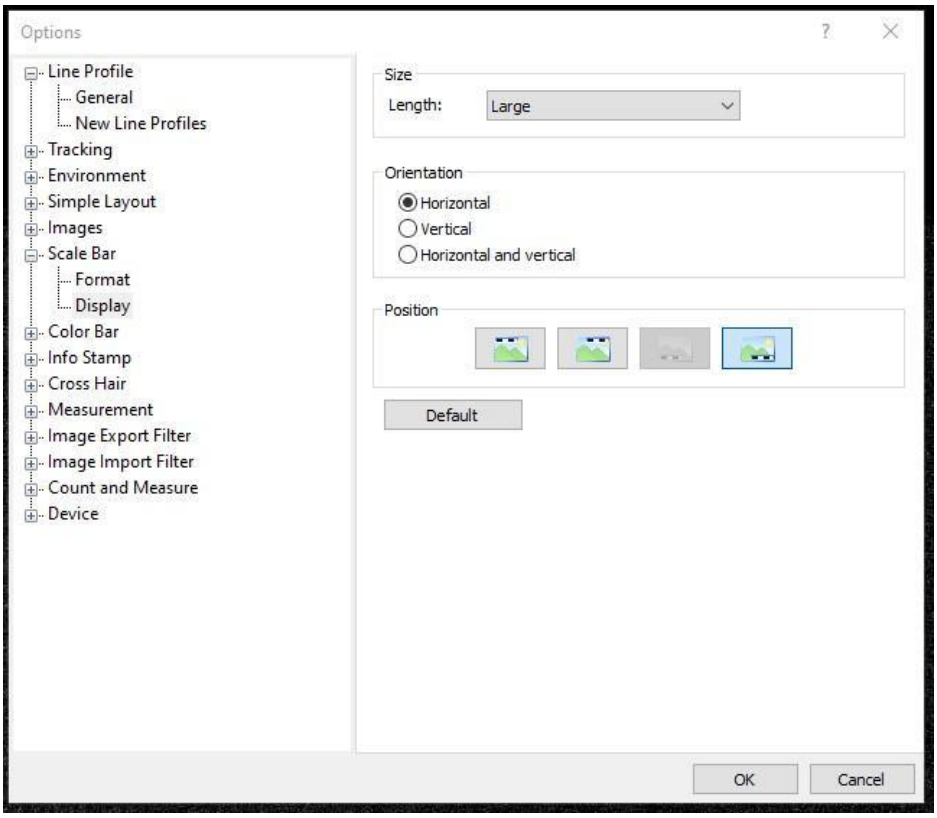
f. To add or remove buttons from the **Camera Control** Tool Window

i. Click  – located at the top-right corner of tool window



3. Set up the display options for live images.
    a. Open a live image
        i. Select **Live** in **Camera Control** Tool Window
        ii. **Acquire** > **Live**
    b. Set *Format* and *Display Options* for Scale Bar: **Tools** > **Options** > **Scale Bar**
        i. Set opacity to 100% or leave *Transparent background* box unchecked
        ii. Set line *Length* to large
        iii. Set *Orientation* to horizontal
        iv. Set *Position* to bottom-right location

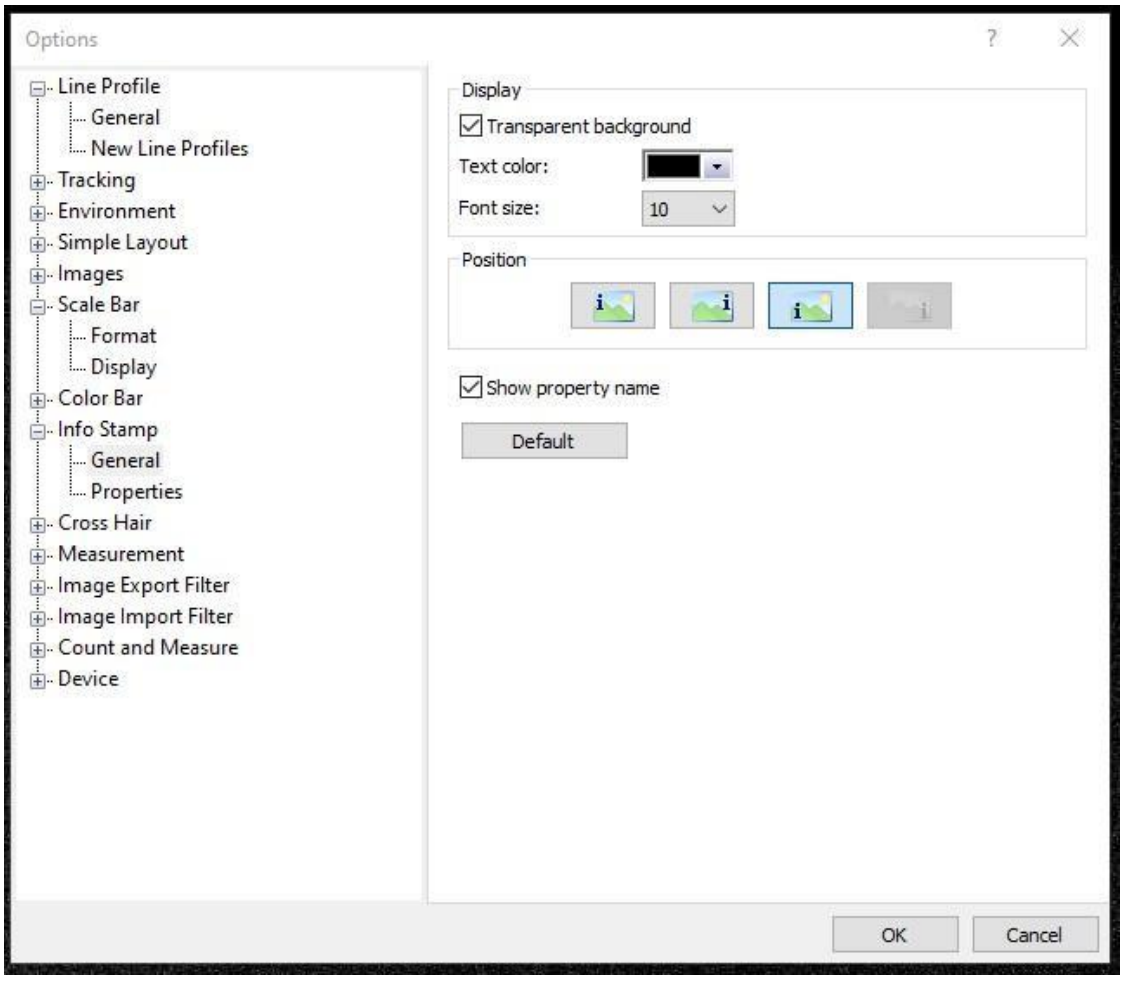

Options
Line Profile
General
New Line Profiles
Tracking
Environment
Simple Layout
Images
Scale Bar
Format
Display
Color Bar
Info Stamp
Cross Hair
Measurement
Image Export Filter
Image Import Filter
Count and Measure
Device
Text style
Show text
Text color:
Font size:
10
Line style
Transparent background
Line color:
Line width:
Medium
Bar:
Use fixed prefix of the image's calibration unit
Default
OK
Cancel


Options
Line Profile
General
New Line Profiles
Tracking
Environment
Simple Layout
Images
Scale Bar
Format
Display
Color Bar
Info Stamp
Cross Hair
Measurement
Image Export Filter
Image Import Filter
Count and Measure
Device
Size
Length:
Large
Orientation
Horizontal
Vertical
Horizontal and vertical
Position
Default
OK
Cancel

c. Set *General* and *Properties Options* for *Info Stamp*:

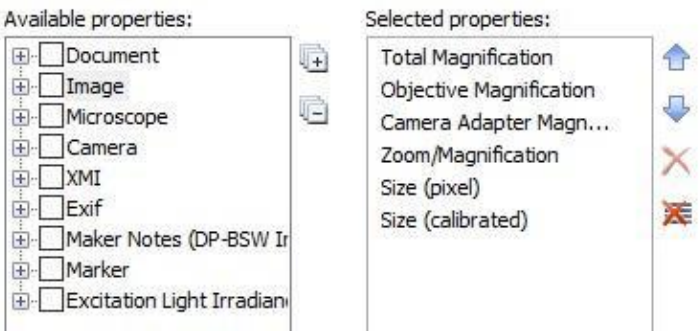
i. **Tools** > **Options** > **Info Stamp** > **General**

- Check *Transparent background* box
- Set *Text color*, *Font size*, and *Position* for *Info Stamp*



ii. **Tools** > **Options** > **Info Stamp** > **Properties** > **Image and Microscope**

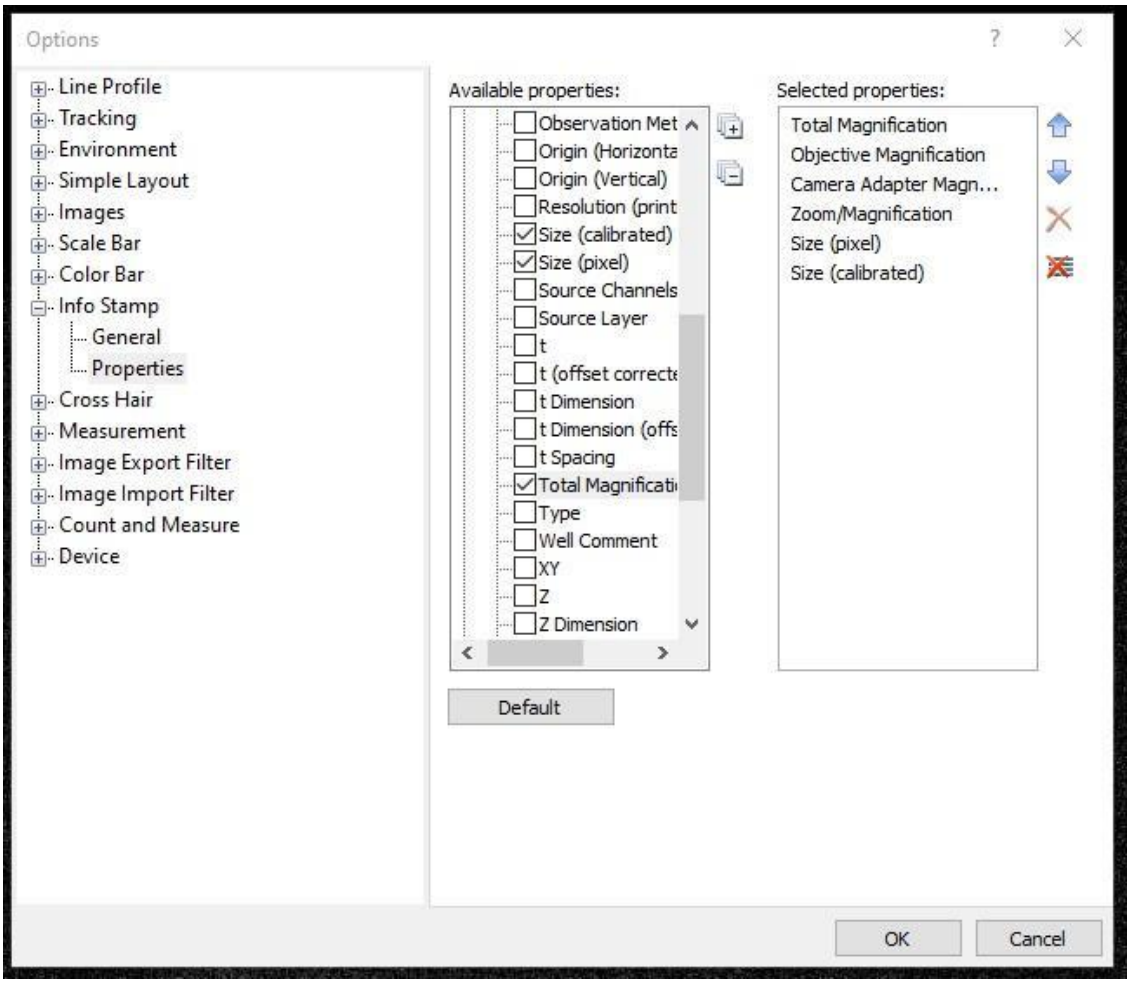
- Options are available for multiple settings



- Select the following *Available properties* for *Image* and *Microscope*:

- *Image*: Total Magnification, Size (calibrated and pixel)
- *Microscope*: Camera Adapter, Objective Magnification, and Zoom/Magnification

Available properties for *Image*



Info Stamp examples:

Total Magnification:	**2.5 x**
Objective Magnification:	**4 x**
Camera Adapter Magnification:	**0.5 x**
Zoom/Magnification:	**1.25 x**

Info Stamp with minimal information

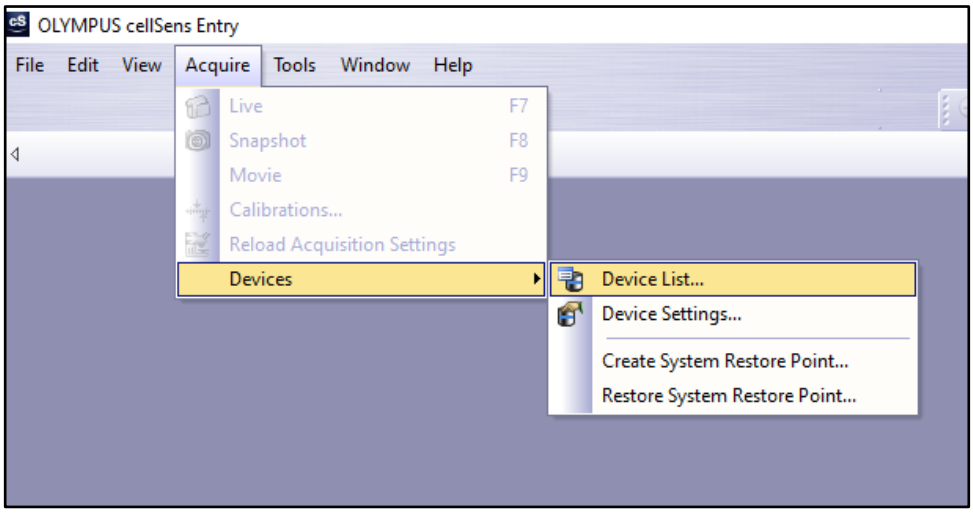
Total Magnification:	**2.5 x**
Objective Magnification:	**4 x**
Camera Adapter Magnification:	**0.5 x**
Zoom/Magnification:	**1.25 x**
Size (pixel):	**1600 x 1200**
Size (calibrated):	**2.9 mm x 2.2 mm**

Info Stamp includes size of *Live* image

Total Magnification:	**2.5 x**
Objective Magnification:	**4 x**
Camera Adapter Magnification:	**0.5 x**
Zoom/Magnification:	**1.25 x**
Size (pixel):	**4800 x 3600**
Size (calibrated):	**2.9 mm x 2.2 mm**

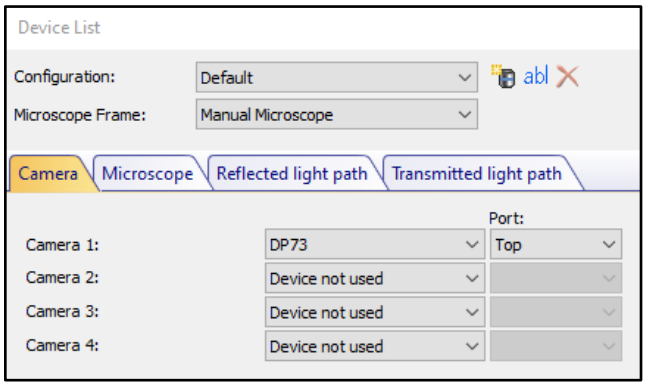
Info Stamp includes size of *Snapshot* image

4. Click **Acquire** > **Devices** > **Device List**



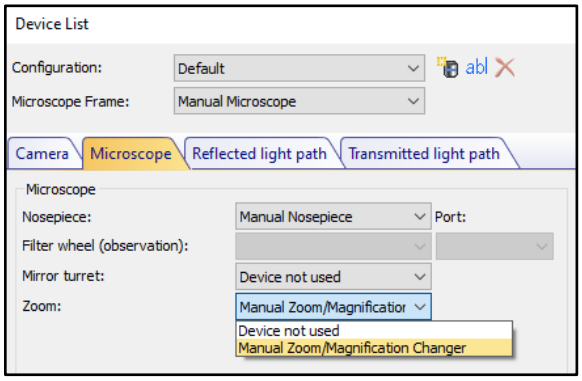
a. **Acquire > Devices > Device List > Camera**

i. Confirm the name of your micro camera is displayed under **Device List Camera**



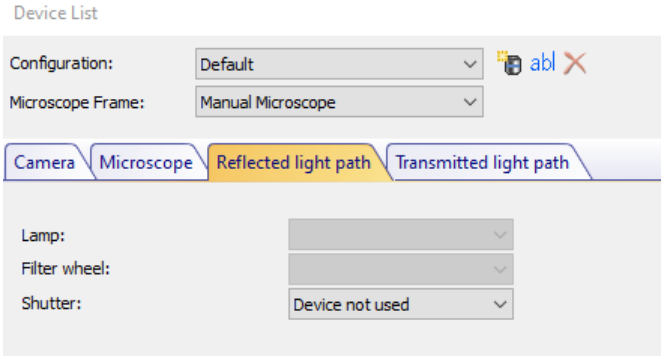
b. **Acquire > Devices > Device List > Microscope**

i. Confirm *Nosepiece* and *Zoom* are set to *Manual Nosepiece* and *Manual Zoom/Magnification Changer* respectively



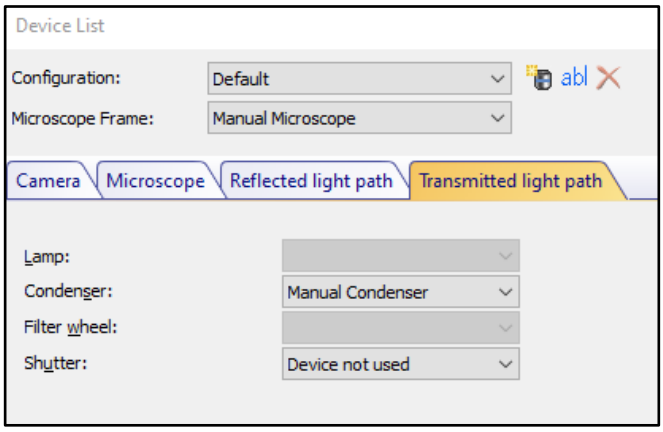
c. **Acquire > Devices > Device List > Reflected Light Path**

i. We found that transmitted light works best for scales:

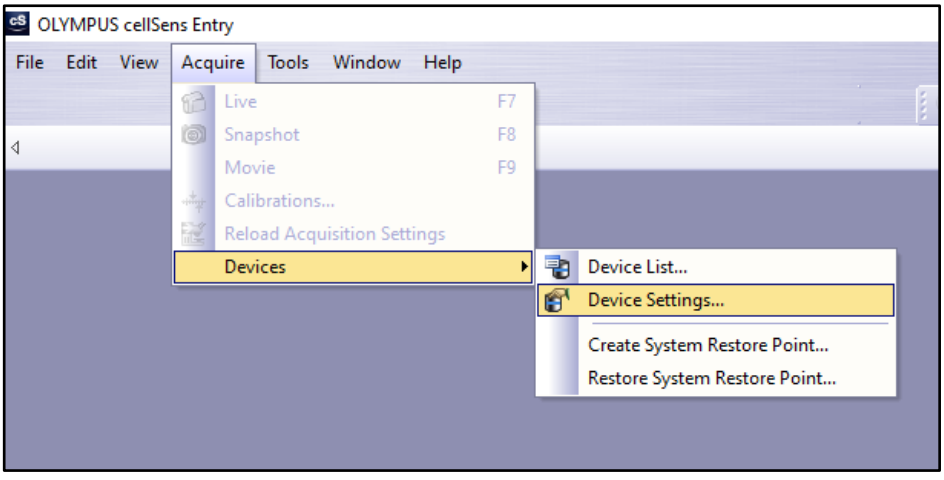


d. **Acquire > Devices > Device List > Transmitted Light Path**

i. Confirm *Condenser* is set to *Manual Condenser*

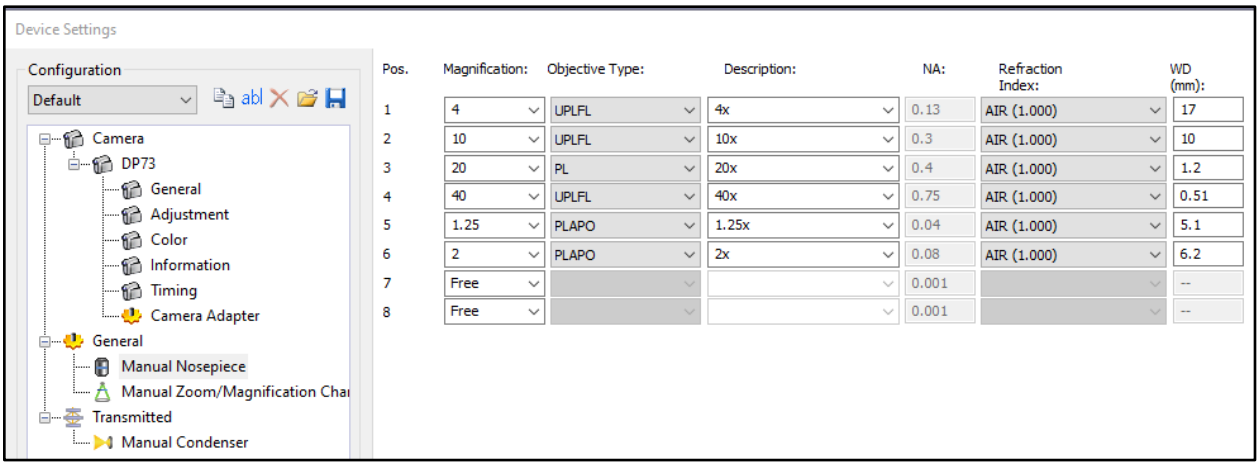


5. Click **Acquire** > **Devices** > **Device Settings…**



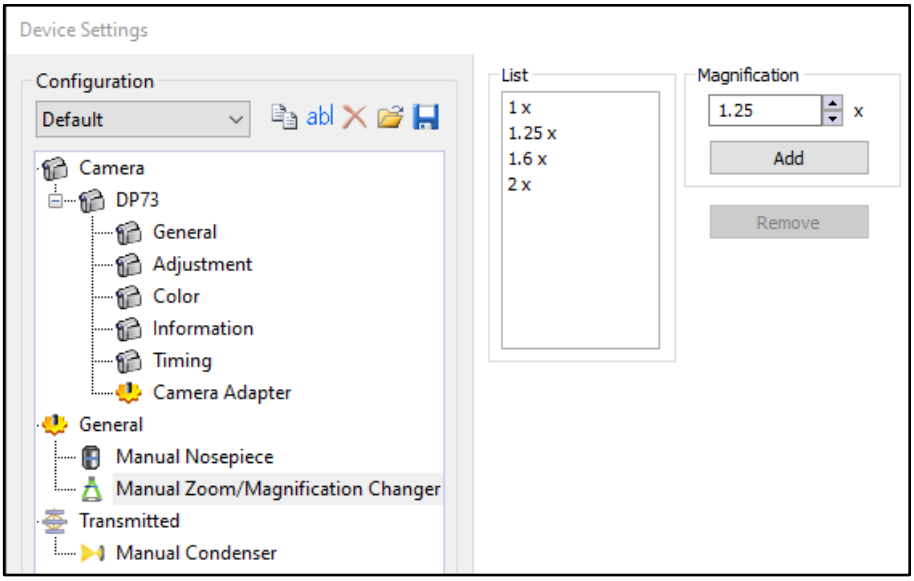
a. **Device Settings > Configuration > General > Manual Nosepiece**

i. Add or remove objective types



b. **Device Settings > Configuration > General > Manual Zoom/Magnification Changer**

i. Add in *Zoom* settings for the *Magnification Changer*. Use the *Magnification* up/down arrows to find desired magnification. Click the **Add** button to insert new magnification to *List*.



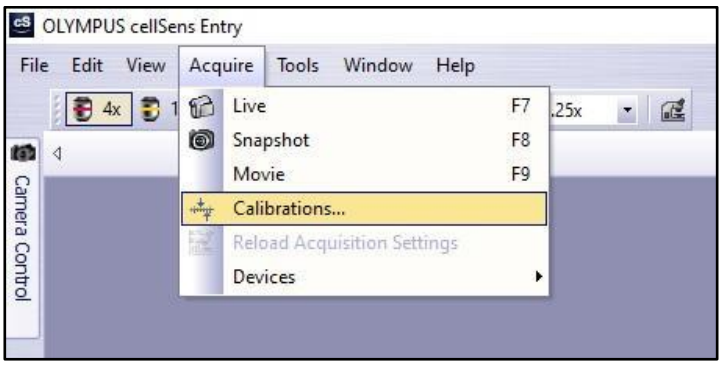
6. Click **Acquire** > **Calibrations**

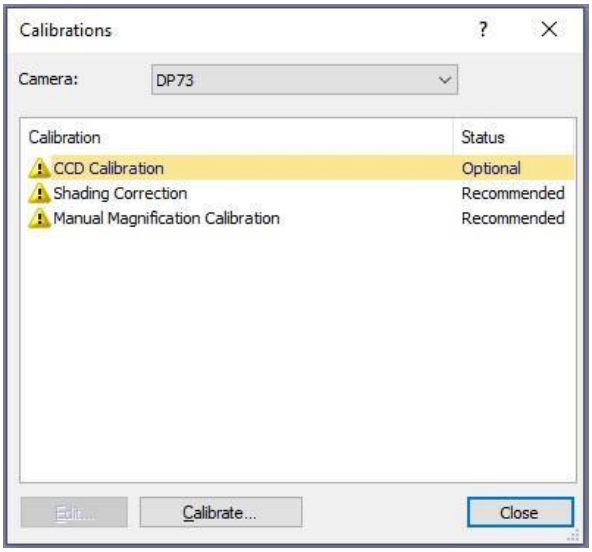
a. The options in the **Calibrations** window vary based upon system capability

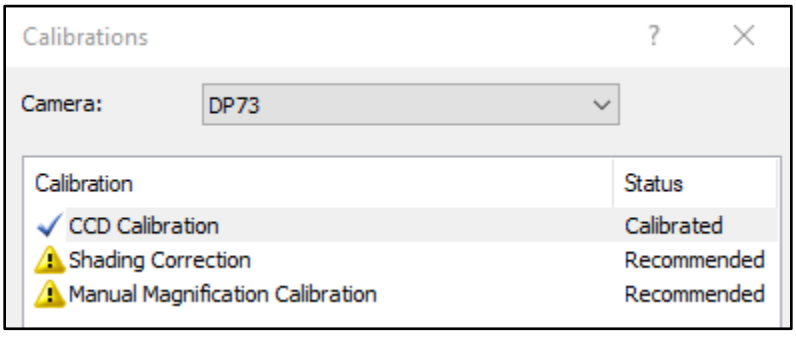
b. Complete recommended and preferred calibrations (see example below)

c. Confirm the status of the desired calibrations before moving on

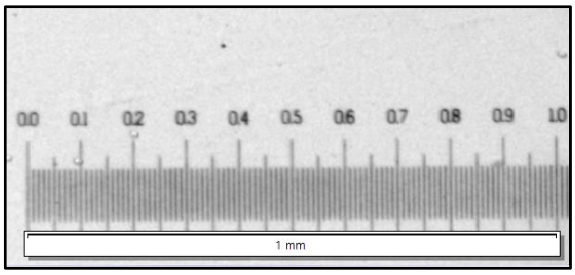
- If you do not complete calibration for all objectives, the icon [⚠] will remain and the status will not read “calibrated,” but the calibrated objectives will be saved.







7. Once complete, confirm calibration settings are correct using a stage micrometer and the scale bar with a live image (**Acquire** > **Live**).

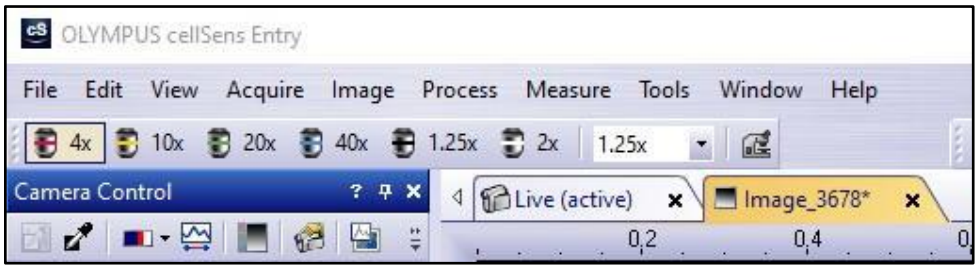


CellSens capturing and saving images (post-setup)

1. Open cellSens
2. Select appropriate *Layout* (top-right)
3. **Acquire** a live image 

4. Place slide with scales to be imaged on microscope stage, and select and focus on a good scale (keep the envelope in front of you, separate from the collection, to avoid mix-up and enable confirmation of the JoinID)
5. Select the largest allowable magnification that captures the entire scale
6. Avoid over focusing. May need to slightly blur the image to produce solid circuli. See example images in the **Aging** section (page 9) of this document.
7. Click the **Snapshot** button when you are ready 

8. Select appropriate *Image Calibration* – objective and zoom



9. CellSens switches to *Live* image. Because of this, you need to select the tab with your saved image. Then, from **Image Menu**, select **Burn in Info** to burn the scale bar into the image. **Note:** the overlay of info is visible on the burned-in info.
10. Save image with JoinID_scaleID and scale location (on the slide, i.e., b2 is the second scale in the second row on the slide). Copy and paste JoinID from data file for filename to minimize transcription errors.
11. Save image with burned in info. **Note:** unsaved image has *in tab.
    [Burn-in saves overlays that are selected in the **View** menu]
12. Select the **Live** tab and move to the next scale. [Image a minimum of three scales per slide, if possible. If scales appear challenging, image more.]
13. Once all scales on the slide are imaged, close all of the saved images so that only the **Live** window is open
14. Continue with the next slide

Tips

1. Once the image is saved, do not close the window. Keep image windows open until your session is ended or you are ready to review. Crosscheck image files with envelopes as you close each window to ensure you actually saved each image.
2. Keep the slide near the envelope. Keep one envelope out at one time to avoid mix ups.
3. If no scales on a slide are usable, check the envelope for more scales that can be mounted.

SCALE MEASURING PROCEDURES

Procedures are applicable for specific software versions; Image-Pro 10 and ImageJ 1.54

ICES standard scale feature placements

In 2011, the International Council for the Exploration of the Sea hosted a scale reading workshop with the goal of facilitating a uniform approach for scale measurements. Growth markers and increment definitions are based on this scale reading workshop (ICES 2011).

Annulus

One year of growth (annual scale segment) is composed of two seasonal growth segments in the scale, a band of relatively widely spaced circuli representing summer growth, followed by a band of relatively tightly spaced circuli representing winter growth. We consider the last circulus of the winter band as marking the annulus. The transition from one annual scale segment to the following annual scale segment is identified based on two metrics:

- The first continuous circulus following the last incomplete circulus in the previous year's winter band;
- Identified by increased growth from narrow- to wider-spaced circuli

We weigh the second metric, based on circuli spacing, more heavily than the first continuous circulus.

Plus-growth

The scale segment that begins after the last annulus and extends to the edge of the scale.

Run-out

Scale segment that begins after the last freshwater annulus and extends to the first marine circulus. Assumed to contain freshwater and marine growth.

First Marine Circulus

Loosely identified by the increased circuli spacing representing sea entry. Can be difficult to determine due to ambiguity in circuli spacing during freshwater to marine transition.

First Summer Maximum

The First Summer Maximum circulus falls between the first marine circulus and the start of the first winter band and is identified as the outer circulus of the circuli pair with the maximum spacing within the group of five greatest intercirculi spacings (rolling average).

Second Summer Maximum

The Second Summer Maximum circulus is identified in the same manner as the First Summer Maximum but occurs after the first winter band.

First Winter Minimum

The First Winter Minimum circulus is identified as the outer circulus of the circuli pair with the minimum spacing within the group of 5 smallest intercirculi spacings (rolling average), and occurs more than 10 circuli from the start of marine growth.

Second Winter Minimum

The Second Winter Minimum circulus is identified in the same manner as the First Winter Minimum but occurs after the second marine summer band.

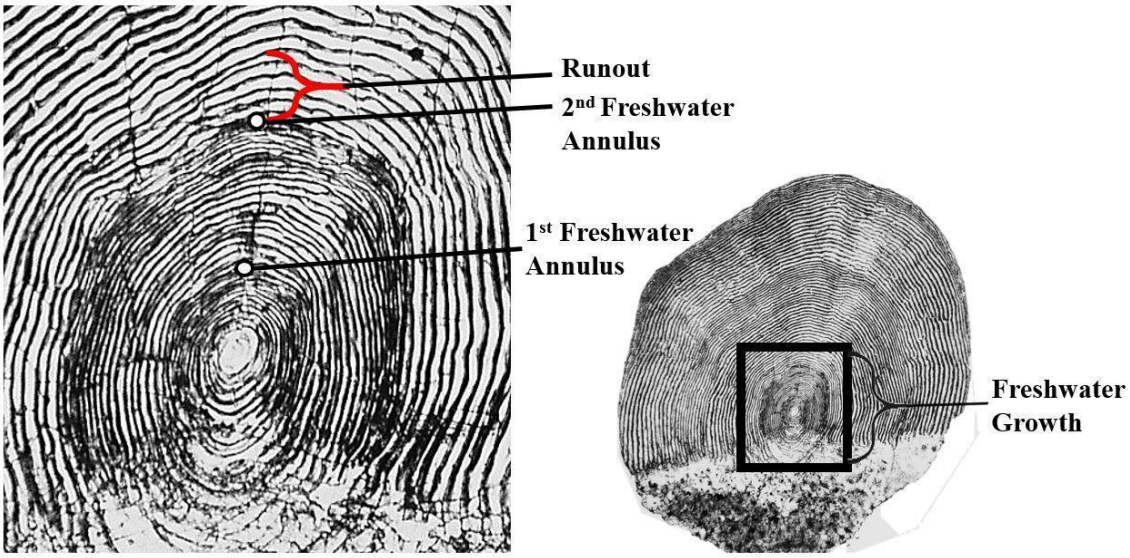


Image of freshwater zone on adult scale and run-out, which can lead to difficulty determining the first marine circulus. *Image from Ellingson et al., 2024*

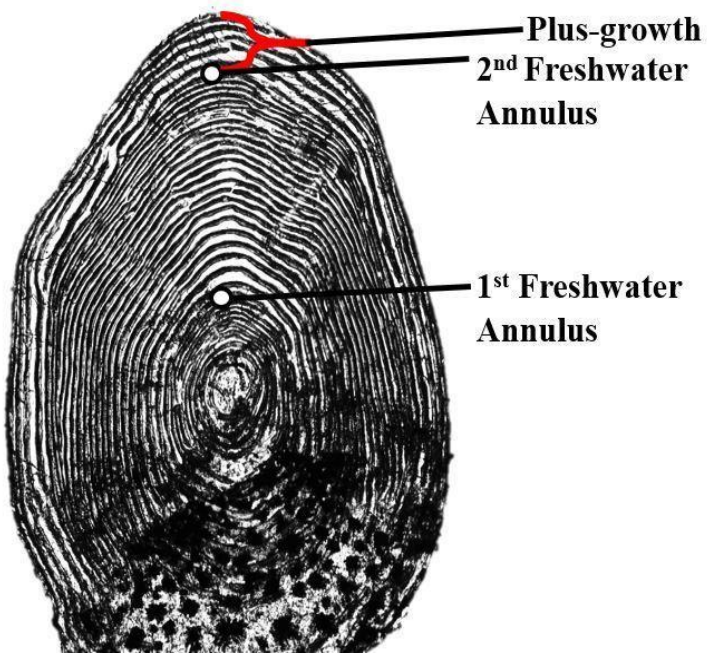
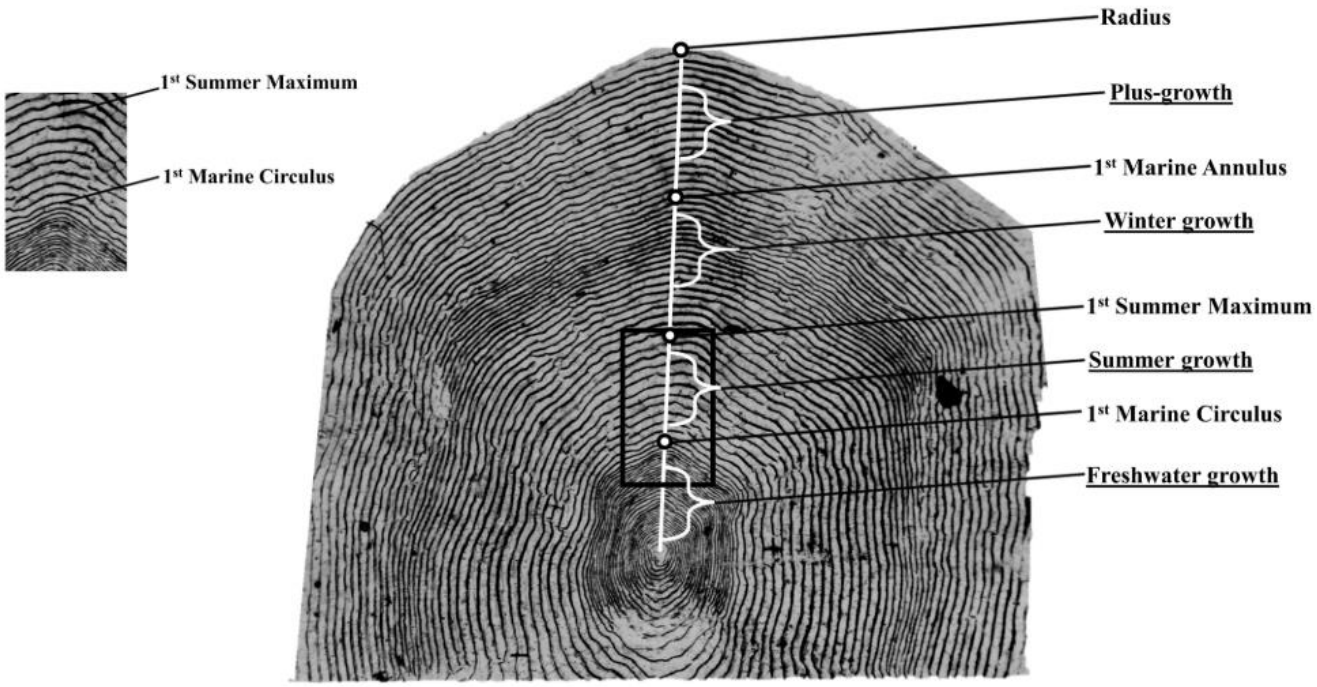


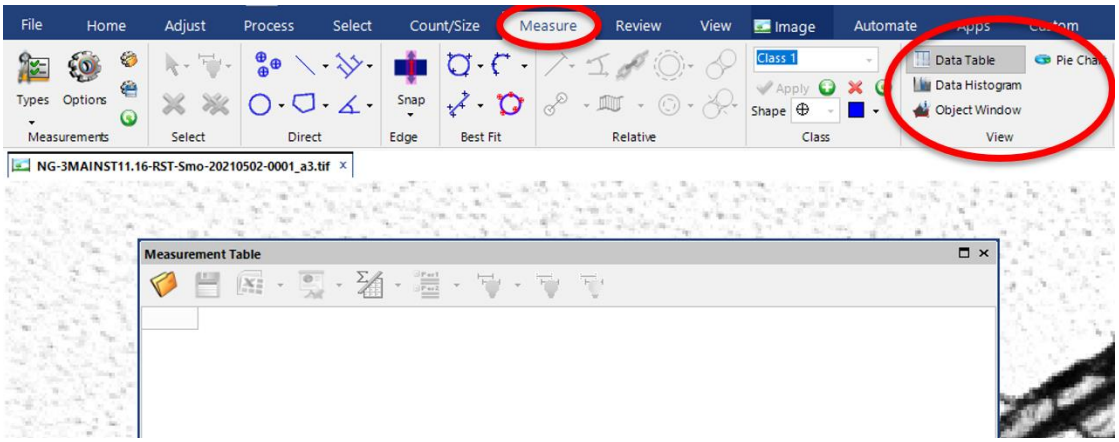
Image of freshwater zone on smolt scale with plus-growth. *Image from Ellingson et al., 2024*



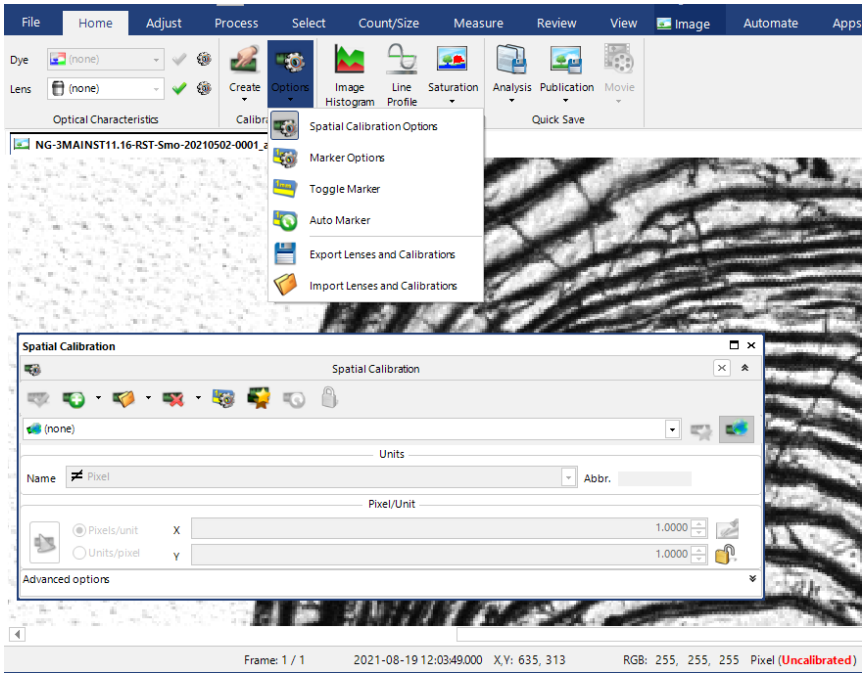
Scale from one sea-winter non-maturing adult sampled at West Greenland. *Retrieved from Tillotson et al., 2021*

Image-Pro setup

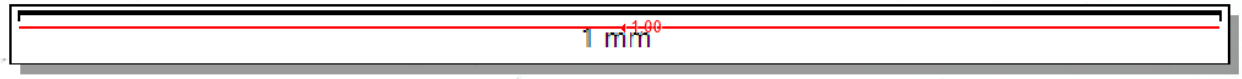
1. Open Image-Pro, then open a scale image
2. Click the **Measure** tab, then in the **View** ribbon, click **Data Table** (This opens the *Measurement Table*)
3. Move *Measurement Table* to second monitor, if present, otherwise move out of Image-Pro window



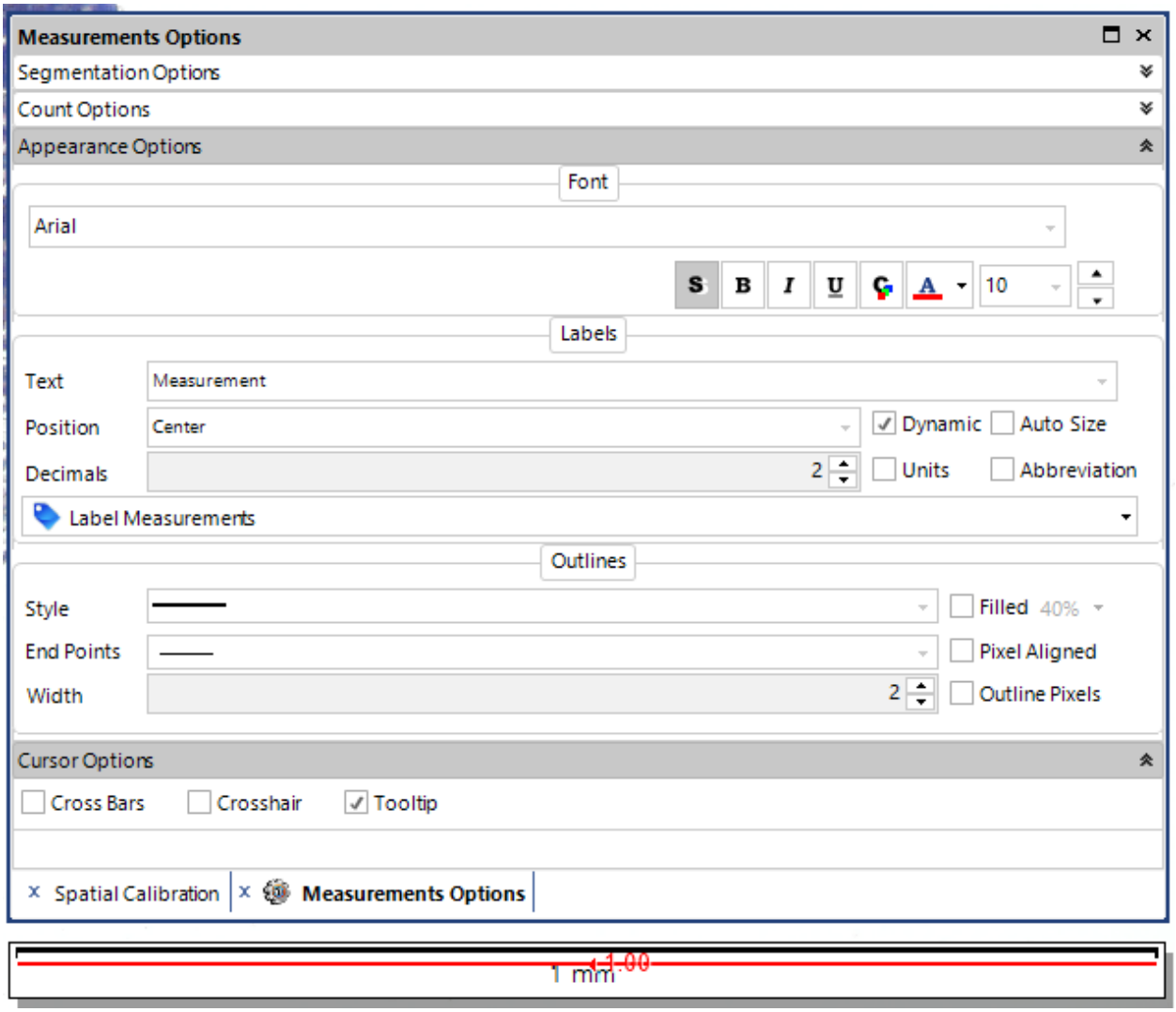
4. Click the **Home** tab (or **Capture** tab depending on the version), then select the **Spatial Calibration Options** tool [ ]
    a. OR, click the *Uncalibrated* [Pixel (Uncalibrated)] bottom-right of window
    b. OR, use HELP – Creating a Quick Calibration



5. If calibrations were previously created, simply select the appropriate calibration from the drop-down menu, then click the green check mark [ ], and go to Step 10.
6. If needed, create a new calibration by selecting **New** tool [ ] at the top of the *Spatial Calibration* window
    a. Type over the default name "Spatial Cal" with a more meaningful one
    b. In the *Unit* area, select the units you want the pixel measurements converted to
    c. In the *Abbr.* text box, enter the abbreviation for the selected units
7. Click the **From Image** button [ ] in the **Pixel/Unit** area.
    a. A dialog box and a horizontal annotation line appear in the image
    b. Click and drag the line to match the length of the scale bar
    c. In the *Reference* length (units) box, type the known length of the scale bar
    d. Click **OK**
    e. The *Spatial Calibration* dialog box is redisplayed and the calculated pixels/unit value appears in the X and Y spin boxes.



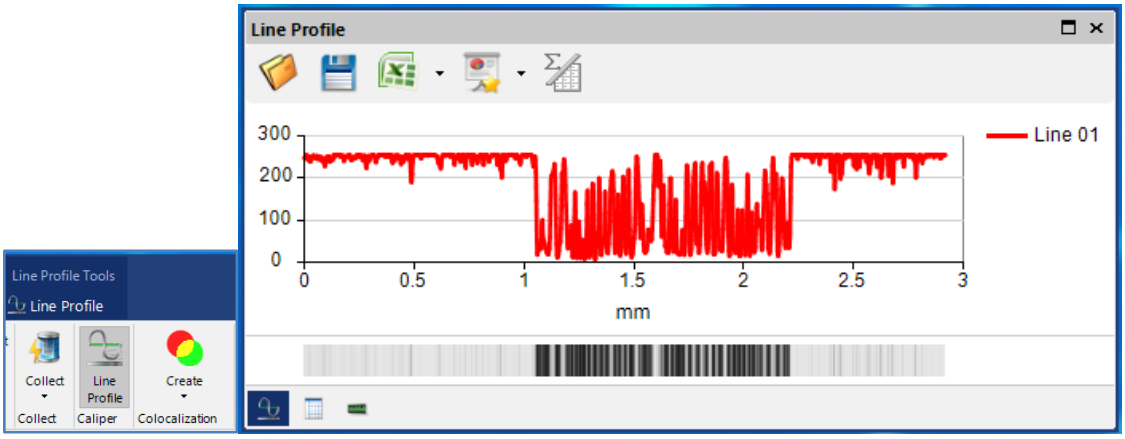
8. Apply newly created calibration to active image by clicking green check mark [ ]
9. Verify the calibration has been applied to the image, bottom-right of window
10. Test the new or previous calibration before use
    a. Click the **Measure** tab
    b. Select the **Line** tool on the **Direct** ribbon
    c. Draw a line to carefully match scale bar, zooming in on the scale bar
    d. On the **Measure** tab, click **View**, then select **Data Table** to see the length of your line
        i. To have the line display the measurement as a label instead of the line name, go to the **Measure** tab and click **Options**. A window will pop up on the right. Under *Appearance Options* (you may need to scroll down or enlarge the window to see it) in the *Text* field, select **Measurement**.



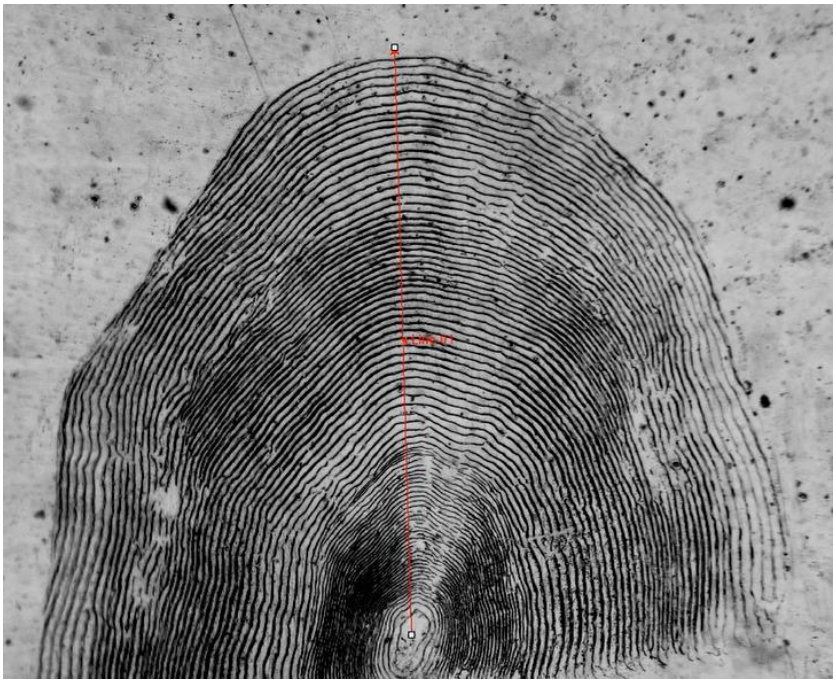
e. Leave the line on the image for verification of calibration on scale measurement overlays

Image-Pro transect measurements setup

1. After calibrating the image to the embedded scale bar, click to the **Measure** tab and select the **Line Profile** tool on the *Caliper* group. A line generates on the image. The **Line Profile Tools** tab appears and a **Line Profile** window opens with three line profile view options: **graph**, **table**, and **measurements** (select from icons in bottom-left of window).

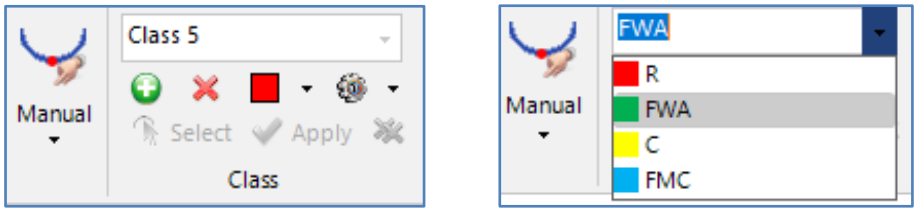


   a. Move the **Line Profile** window to secondary monitor, if possible.
   b. Drag the line on the image to the transect location, with the line origin in the center of the focus extending beyond the scale edge, along the longest axis.
      i. Optionally, you may delete [ ] the pop-up line and draw a new line using the line tool [ ]

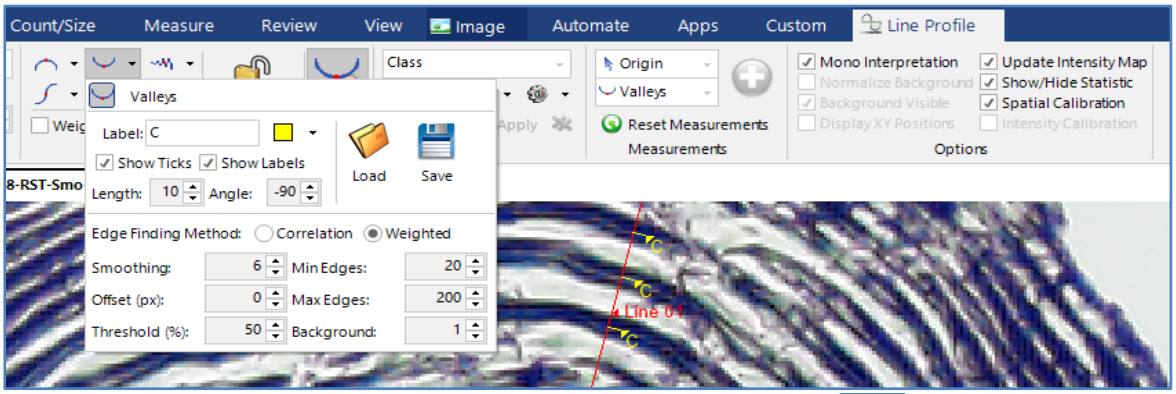


2. For scale features (annulus, circuli, etc.) – add classes for flagging on transect:

a. In the **Class** group, select the drop-down menu and rename the feature to the desired scale feature
b. Select colors that will be visible on image
c. Click the **add Class** icon [ ]
d. Repeat until all scale features are present in class drop-down menu
e. Ensure these steps are followed in order, or an error message will appear
f. In the **Class** group, click the settings icon [ ] and make sure *Show Class Name on Image* is checked
    i. If not checked, the label and color from the **Valleys** settings drop-down menu will be used for all classes



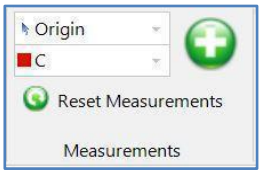
3. For automated detection of circuli:
    a. In the **Class** group, select the circulus scale feature
    b. In the **Edges** group, select the **Valleys** tool 
    c. Use the drop-down arrow to select **Settings**, and ensure the label name and color match the class created for circuli
    d. *Threshold* and *Smoothing* can be adjusted to increase accuracy of circulus detection
    e. In the **Options** group, select **Mono Interpretation**, so that only one set of measurements generates
    f. In the **Valleys** tool, select a color that is visible on your image
    g. Click the **Valleys** button to add points to your center line



    h. Edit the circuli edges using the **Manual Valleys** tool 

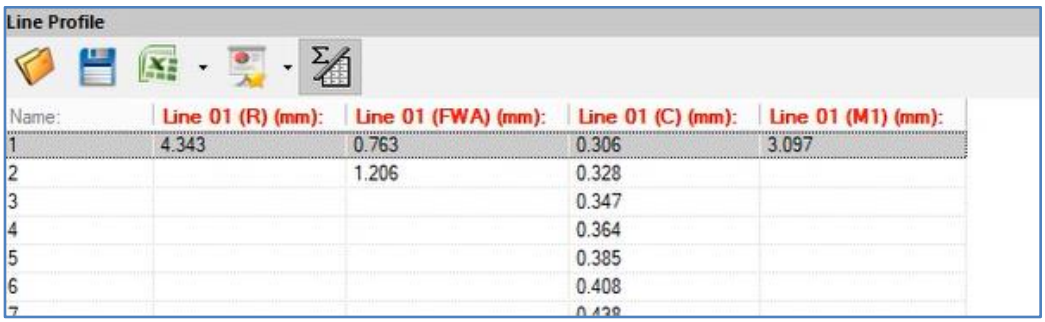
1. Left-click over the location you want to place a marker
2. To remove, Shift + Left-click

4. Once all classes are created, they must be added to the line profile output table
    a. In the **Measurement** tab, above **Measurements**, the user must select **Origin** for the top drop-down menu and then the desired scale feature for the lower drop-down menu



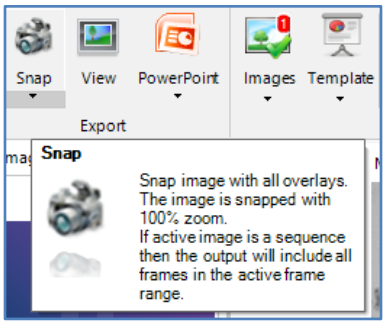
    b. Click the green plus sign to add feature to *Line Profile* output table
    c. Repeat for each scale feature
    d. Verify *Line Profile* output table has desired information
        i. Right-clicking the column names in the *Line Profile* output table will allow for the removal of unwanted columns or features from the table

5. Once measurements have been completed, *Line Profile* output must be exported to Excel



    a. In **Line Profile** output table, select drop-down menu next to the Excel icon
        i. Click **Excel Export Options** and verify the following settings: *export to selected/active excel sheet, append to the right, copy image name to cell*
    b. Place cursor in the open Excel file
    c. Click the **Export Excel** icon
    d. Verify data has been exported to the Excel file

6. After data has been exported, an overlay of the measurement transect must be saved
    a. Click the **Review** tab
    b. In the **Export** group, click on the **Snap** icon



c. Save image as a .jpeg

Review of processing steps for Image-Pro 10

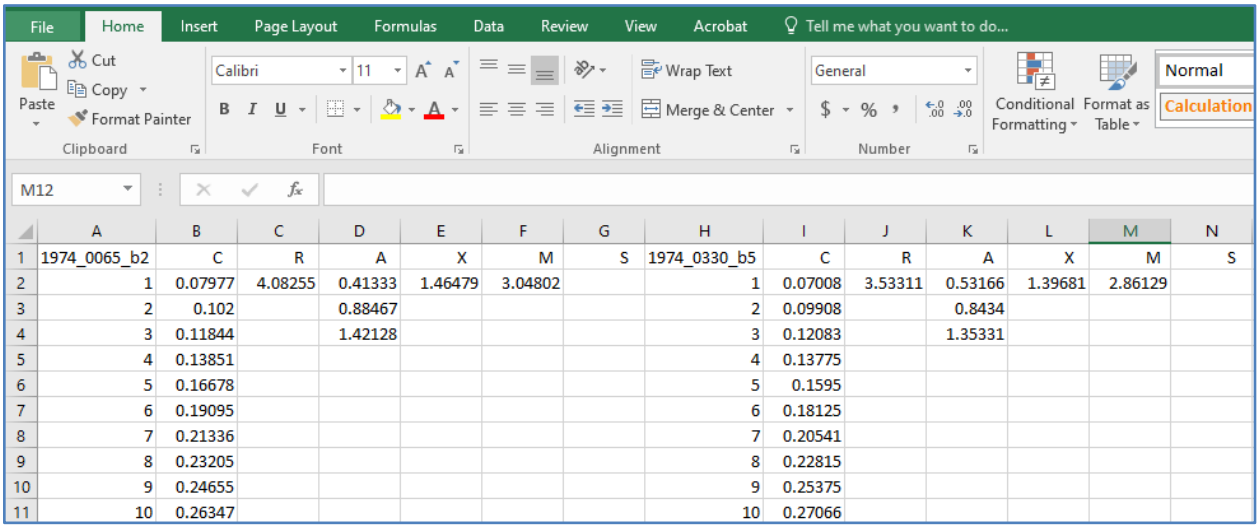
Calibration and line profile settings must be setup prior to use

1. Open image.
2. Verify the calibration window and measurement table are on the second monitor.
3. Select calibration window.
4. Apply appropriate calibration.
5. Test calibration with line measurement tool.
6. Select Line Profile.
7. Place transect along the longest axis.
8. Select desired class (radius, annuli, etc.).
9. Click on transect at desired location to add class marker.
10. Repeat for each class, using Edge Finder last for C.
11. Confirm all data is present in the line measurement table.
12. In the open Excel file, place the cursor in the target cell.
13. Select **export to Excel**.
14. Save Excel.
15. Snap Image overlay and save.
16. Repeat with the next image.

Measurement data formatting

The following steps are required to convert the raw measurement output from Image-Pro 10 or ImageJ 1.54 into the standard format used for scale measurement data.

1. Confirm raw measurement data have been exported properly



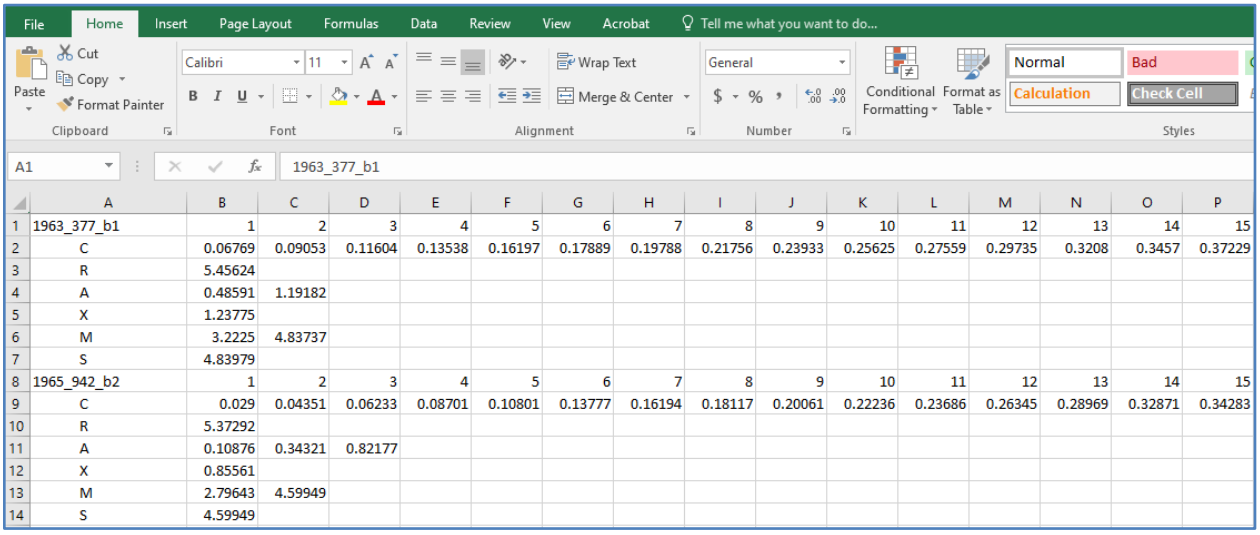
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	1974_0065_b2	C	R	A	X	M	S	1974_0330_b5	C	R	A	X	M	S
2	1	0.07977	4.08255	0.41333	1.46479	3.04802		1	0.07008	3.53311	0.53166	1.39681	2.86129	
3	2	0.102		0.88467				2	0.09908		0.8434			
4	3	0.11844		1.42128				3	0.12083		1.35331			
5	4	0.13851						4	0.13775					
6	5	0.16678						5	0.1595					
7	6	0.19095						6	0.18125					
8	7	0.21336						7	0.20541					
9	8	0.23205						8	0.22815					
10	9	0.24655						9	0.25375					
11	10	0.26347						10	0.27066					

2. Image-Pro 10 will generate summary statistics for each individual that must be deleted
    a. ImageJ does not generate these unless specified, ImageJ users can skip this step

81	80	3.30518						80	2.7507					
82	81	3.40179						81	2.79639					
83	82	3.47619						82	2.82574					
84	83	3.54388						83	2.85789					
85	84	3.60665						84	2.88876					
86	85	3.68256						85	2.9268					
87	86	3.74471						86	2.96065					
88	87	3.80974						87	3.00018					
89	88	3.87502						88	3.06248					
90	89	3.94308						89	3.11886					
91	90	4.01245						90	3.16839					
92	Min	0.07977	4.08255	0.41333	1.46479	3.04802	0	91	3.22394					
93	Max	4.01245	4.08255	1.42128	1.46479	3.04802	0	92	3.2957					
94	Mean	1.6568	4.08255	0.90643	1.46479	3.04802	0	93	3.36913					
95	StdDev	1.16788	0	0.41178	0	0	0	Min	0.07008	3.53311	0.53166	1.39681	2.86129	0
96	Samples	90	1	3	1	1	0	Max	3.36913	3.53311	1.35331	1.39681	2.86129	0
97	Sum	149.1121	4.08255	2.71928	1.46479	3.04802	0	Mean	1.41631	3.53311	0.90946	1.39681	2.86129	0
98								StdDev	0.98877	0	0.33867	0	0	0
99								Samples	93	1	3	1	1	0
100								Sum	131.7171	3.53311	2.72837	1.39681	2.86129	0

**This step can be done manually or by using the Excel macros provided below*

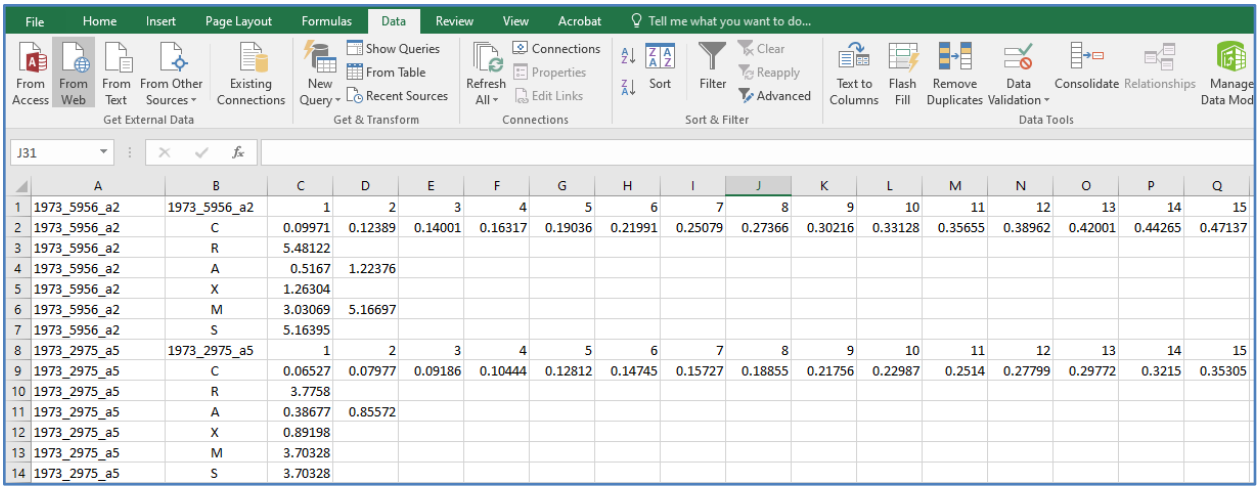
3. Measurement data must be transposed
    a. Highlight all measurement data, and paste special (transpose) into new excel sheet



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	1963_377_b1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	C	0.06769	0.09053	0.11604	0.13538	0.16197	0.17889	0.19788	0.21756	0.23933	0.25625	0.27559	0.29735	0.3208	0.3457	0.37229
3	R	5.45624														
4	A	0.48591	1.19182													
5	X	1.23775														
6	M	3.2225	4.83737													
7	S	4.83979														
8	1965_942_b2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
9	C	0.029	0.04351	0.06233	0.08701	0.10801	0.13777	0.16194	0.18117	0.20061	0.22236	0.23686	0.26345	0.28969	0.32871	0.34283
10	R	5.37292														
11	A	0.10876	0.34321	0.82177												
12	X	0.85561														
13	M	2.79643	4.59949													
14	S	4.59949														

**Transposed data should look like this*

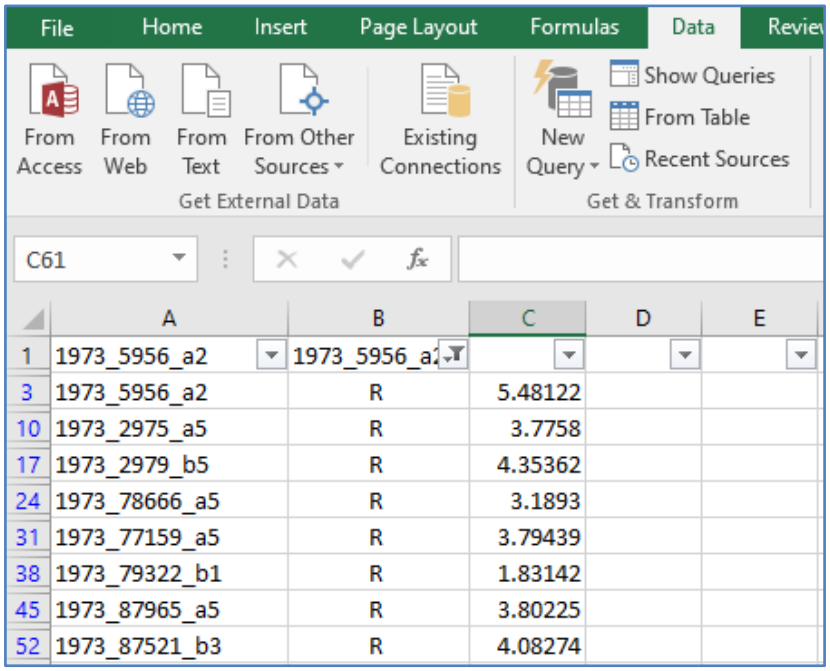
4. Create a new column to the left of the spreadsheet and copy paste the individual ID to each of the corresponding rows that contain measurement data for that fish



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	1973_5956_a2	1973_5956_a2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	1973_5956_a2	C	0.09971	0.12389	0.14001	0.16317	0.19036	0.21991	0.25079	0.27366	0.30216	0.33128	0.35655	0.38962	0.42001	0.44265	0.47137
3	1973_5956_a2	R	5.48122														
4	1973_5956_a2	A	0.5167	1.22376													
5	1973_5956_a2	X	1.26304														
6	1973_5956_a2	M	3.03069	5.16697													
7	1973_5956_a2	S	5.16395														
8	1973_2975_a5	1973_2975_a5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
9	1973_2975_a5	C	0.06527	0.07977	0.09186	0.10444	0.12812	0.14745	0.15727	0.18855	0.21756	0.22987	0.2514	0.27799	0.29772	0.3215	0.35305
10	1973_2975_a5	R	3.7758														
11	1973_2975_a5	A	0.38677	0.85572													
12	1973_2975_a5	X	0.89198														
13	1973_2975_a5	M	3.70328														
14	1973_2975_a5	S	3.70328														

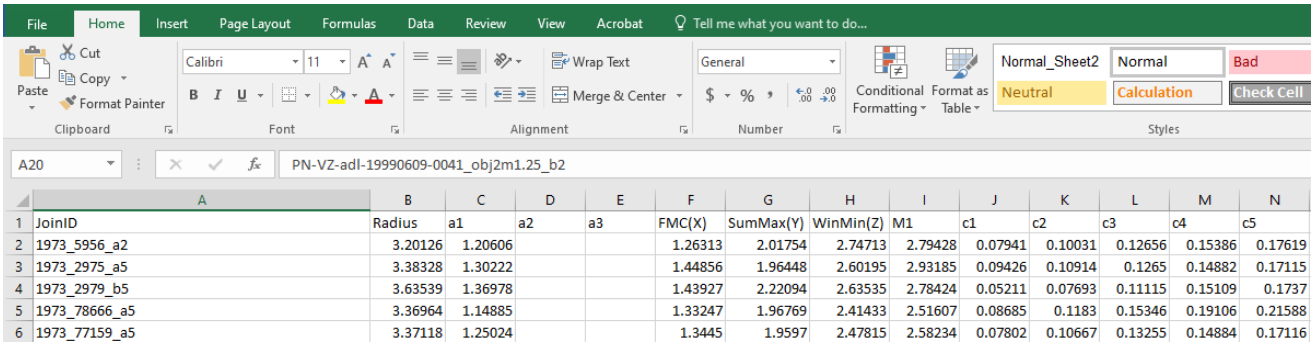
**This can be done manually or by using the Excel macros provided below*

5. Now each scale feature (C - circuli, R - radius, etc.) can be extracted and pasted to another document
    a. Filter by each scale feature using column B
    b. Copy and paste each scale features–associated data (column C) into final spreadsheet



	A	B	C	D	E
1	1973_5956_a2	1973_5956_a2			
3	1973_5956_a2	R	5.48122		
10	1973_2975_a5	R	3.7758		
17	1973_2979_b5	R	4.35362		
24	1973_78666_a5	R	3.1893		
31	1973_77159_a5	R	3.79439		
38	1973_79322_b1	R	1.83142		
45	1973_87965_a5	R	3.80225		
52	1973_87521_b3	R	4.08274		

6. Verify the final format for the measurement data matches the image provided below.
    a. Horizontal orientation of circulus measurements.
    b. Each individual should have all associated data in one row.
    c. Circuli should be the last features listed on the right of the spreadsheet.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	JoinID	Radius	a1	a2	a3	FMC(X)	SumMax(Y)	WinMin(Z)	M1	c1	c2	c3	c4	c5
2	1973_5956_a2	3.20126	1.20606			1.26313	2.01754	2.74713	2.79428	0.07941	0.10031	0.12656	0.15386	0.17619
3	1973_2975_a5	3.38328	1.30222			1.44856	1.96448	2.60195	2.93185	0.09426	0.10914	0.1265	0.14882	0.17115
4	1973_2979_b5	3.63539	1.36978			1.43927	2.22094	2.63535	2.78424	0.05211	0.07693	0.11115	0.15109	0.1737
5	1973_78666_a5	3.36964	1.14885			1.33247	1.96769	2.41433	2.51607	0.08685	0.1183	0.15346	0.19106	0.21588
6	1973_77159_a5	3.37118	1.25024			1.3445	1.9597	2.47815	2.58234	0.07802	0.10667	0.13255	0.14884	0.17116

Macros

Based upon the number of scales being measured, Excel macros are recommended to expedite the formatting process and reduce the occurrence of human error.

Macros can be found on the **Developer** tab in Excel

To add the **Developer** tab: **File > Options > Customize Ribbon >** check **Developer** box

1. On the **Developer** tab: select macro, enter the macro name, then click **Create**
2. Copy and paste the text below to the macro window, then click **File** > **Save**
3. In the **Macros** tab under **Options**, set keyboard shortcuts (Ctrl + t, etc.)

PURPOSE: Used to clear summary statistics – *See step 2 in previous section*

How To Use: Click the macro shortcut repeatedly until all summary statistics have been removed

```
Sub summary_clear()
Cells.Find(what:="Min", after:=ActiveCell, LookIn:=xlValues, LookAt:= _
    xlPart, SearchOrder:=xlByRows, SearchDirection:=xlNext, MatchCase:=False _
    , SearchFormat:=False).Activate
  ActiveCell.Offset(0, 0).Range("A1:F9").Select
  Selection.ClearContents
End Sub
```

PURPOSE: Copies JoinIDs to lefthand column – *See step 4 in previous section*

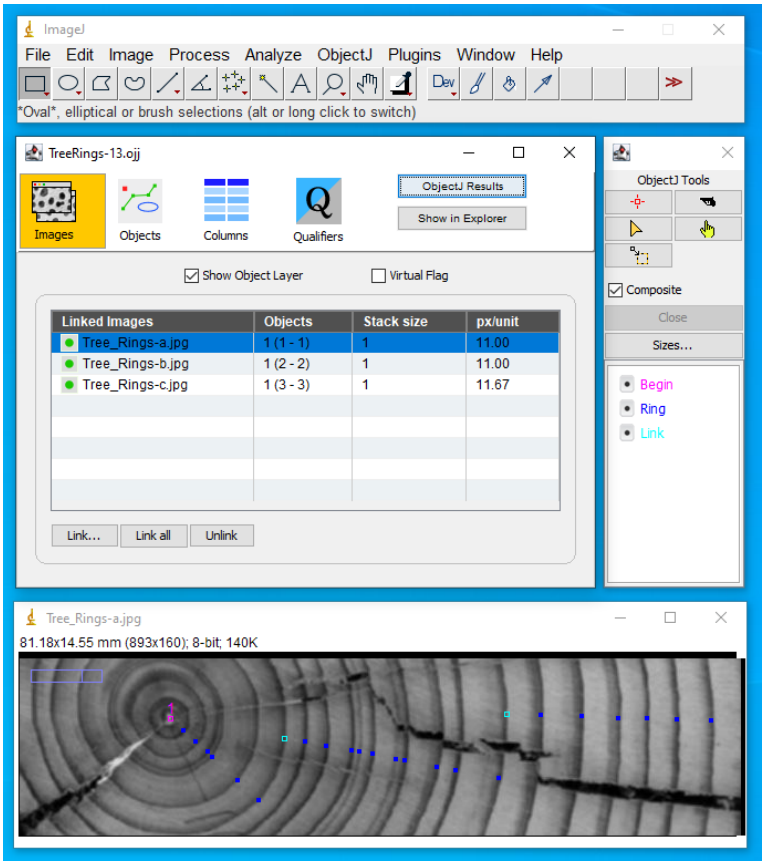
How To Use: Select the cell to the left of the desired JoinID and click the macro shortcut

```
Sub JoinID_copy()
ActiveCell.Offset(0, 1).Range("A1").Select
Selection.Copy
ActiveCell.Offset(0, -1).Range("A1:A7").Select
ActiveSheet.Paste
End Sub
```

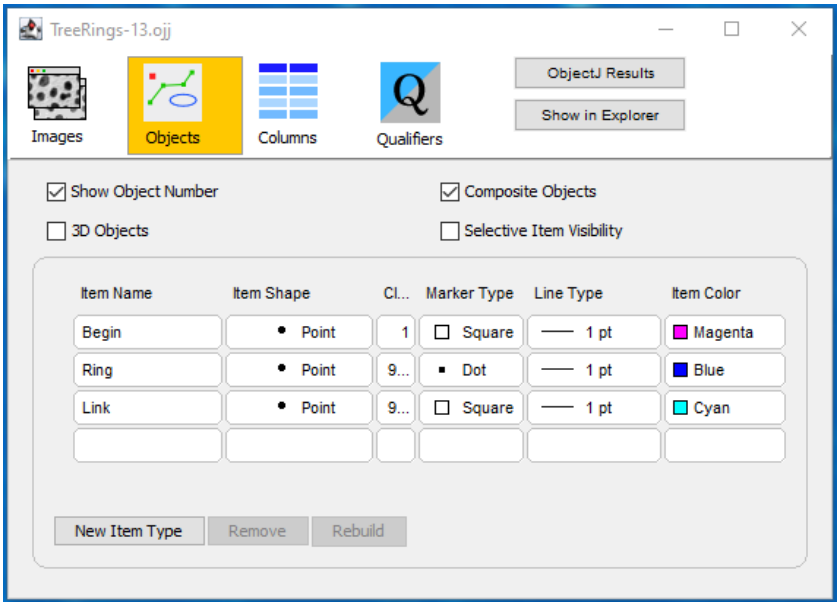
ImageJ setup

ImageJ is a free alternative to Image-Pro that has been shown by ASEB staff to produce comparable results.

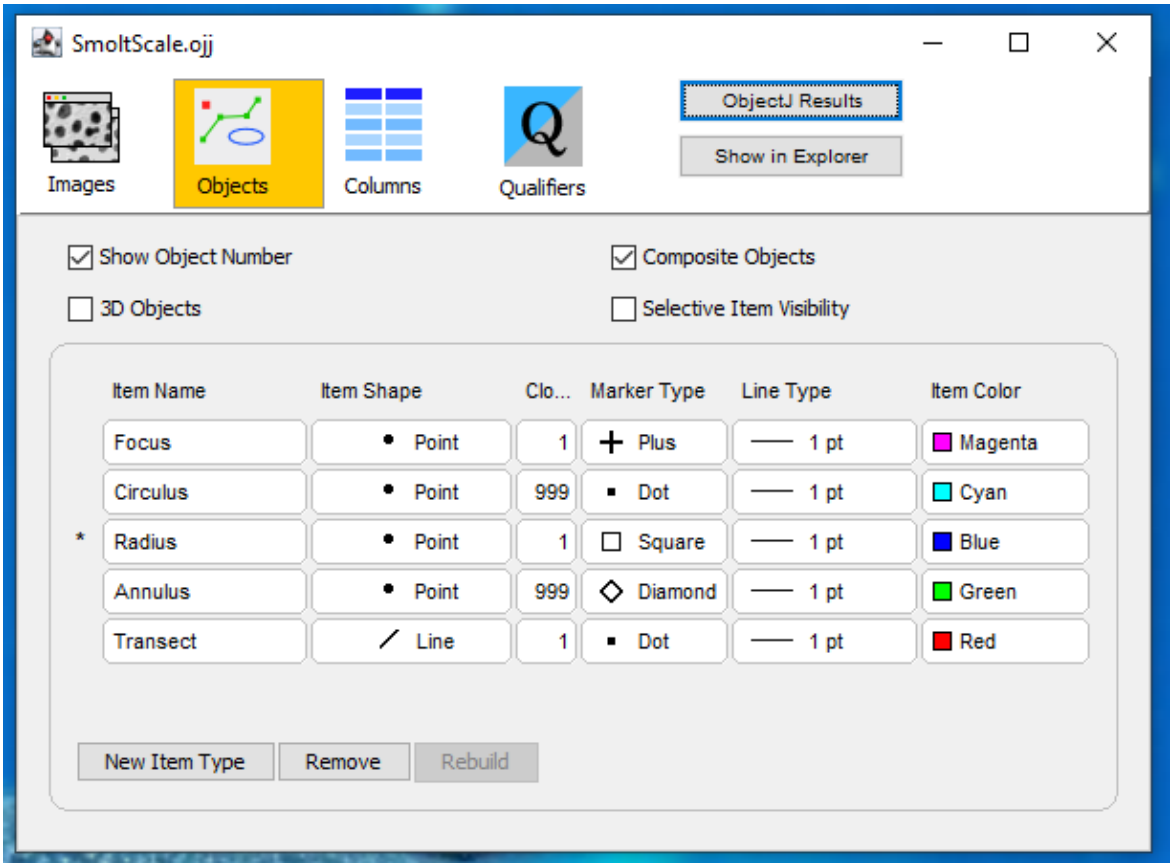
1. Download and unzip ImageJ
    a. https://imagej.net/ij/download.html
2. Download the ImageJ plugin 'objectj_jar' from the **Current** folder
    a. https://sils.fnwi.uva.nl/bcb/objectj/download/
    b. Move *.jar file into ImageJ\Plugins\jars
3. Download and unzip most recent TreeRings macro
    a. https://sils.fnwi.uva.nl/bcb/objectj/examples/TreeRings/TreeRings-9.htm
    b. Rename "TreeRings" folder to something meaningful, such as "ImageJ Projects" This folder will house your projects and images. Move the project folder into the ImageJ folder
4. Open ImageJ **ImageJ.exe** file
5. Select **Plugins** > **jars** > **ObjectJ**
    a. If ObjectJ does not appear near the bottom, verify step 2 was done correctly
6. ObjectJ will now appear in the menu to the left of *Plugins*
    a. Select **ObjectJ**, then **Project** > **Open Project**
    b. Select the **TreeRings-##.ojj** from the downloaded location.
7. After opening the .ojj file, select one of the tree ring images under *Linked Images* in the **TreeRings-##.ojj** window
    a. There should now be four separate windows open
        i. ImageJ, TreeRings-##.ojj, Object J Tools, and Tree_Rings-#.jpg
    b. You may move each window around your desktop for your preferred working layout



8. Now we need to change the tree ring macro for suitability to measure salmon scales
9. Select **Objects** in the "TreeRings-##.ojj" project window
    a. Each measurement feature (Begin, Ring, Link) can be changed and customized for measuring scales



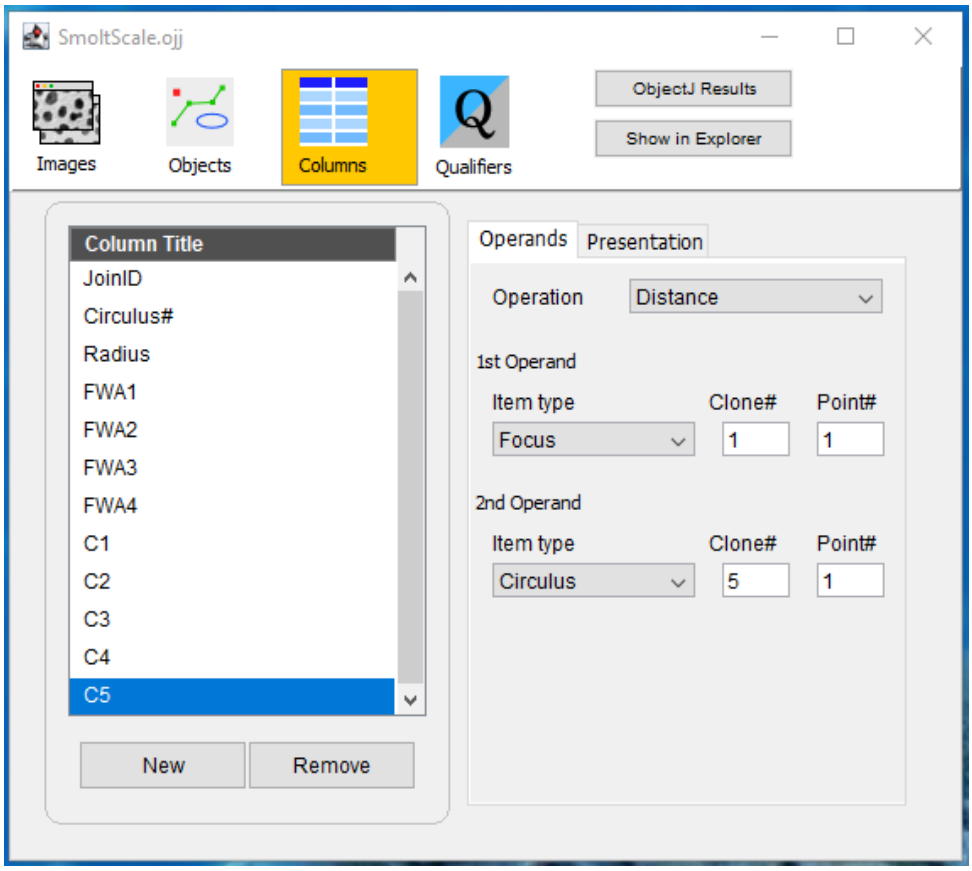
10. Create a new project for salmon scales
    a. **ImageJ window > ObjectJ > Project > New Project** > Name your project
11. Open the new project
    a. **ImageJ window > ObjectJ > Project > Open Project**
12. Add new measurement features
    a. Rename each Item Name as desired (*Annulus*, *Circulus*, *Focus*, *Radius*, etc.) by double-clicking in the *Item Name* box
    b. To add a new item, click *New Item Type*; double-click in *Item Name* and *Item Shape* to add new name and desired shape
    c. Add a new item and name it *Transect*, change the *Item Shape* to *Line* all other *Item Shapes* should be *Points*
    d. Choose *Marker Types* and *Item Colors* to maximize visibility by double-clicking in each box
    e. Set clones values to 1 for *Focus*, *Radius*, and *Transect*
    f. For scale features that will be marked more than once (i.e., circuli and annuli), set clones to 999



13. Select the **Columns** tab in the **TreeRings-##.ojj** project window
    a. Select and remove each *Column Title* for TreeRings (*KnownYear*, *BeginYear*, *EndYear*, *Age*, *File*) by highlighting one at a time and clicking **Remove**

b. Add measurement features for proper export formatting by clicking **New** under the **Column Title** section. Double-click on the new **Column#** title to rename it to a measurement feature.
    i. JoinID (or sample ID): Operand, select **File Name** from Operation list
    ii. Circulus#
        1. Operation, select **Count**
        2. **Operand** > **Item Type**, select **Circulus** from drop-down menu
    iii. Add *Column Titles* for remaining scale features.
        1. Operation for measurement features is *Distance*
        2. First Operand for measurement features should always be *Focus* (clone # = 1, point # = 1)
        3. Second Operand for measurement features is the *Item Type* (i.e., *Circulus* or *Annulus*) that matches the *Object* you have set up. Clone number matches the iteration of the feature (e.g., Clone# = 5, Point# = 1 for C5 - the fifth circulus): see figure below.

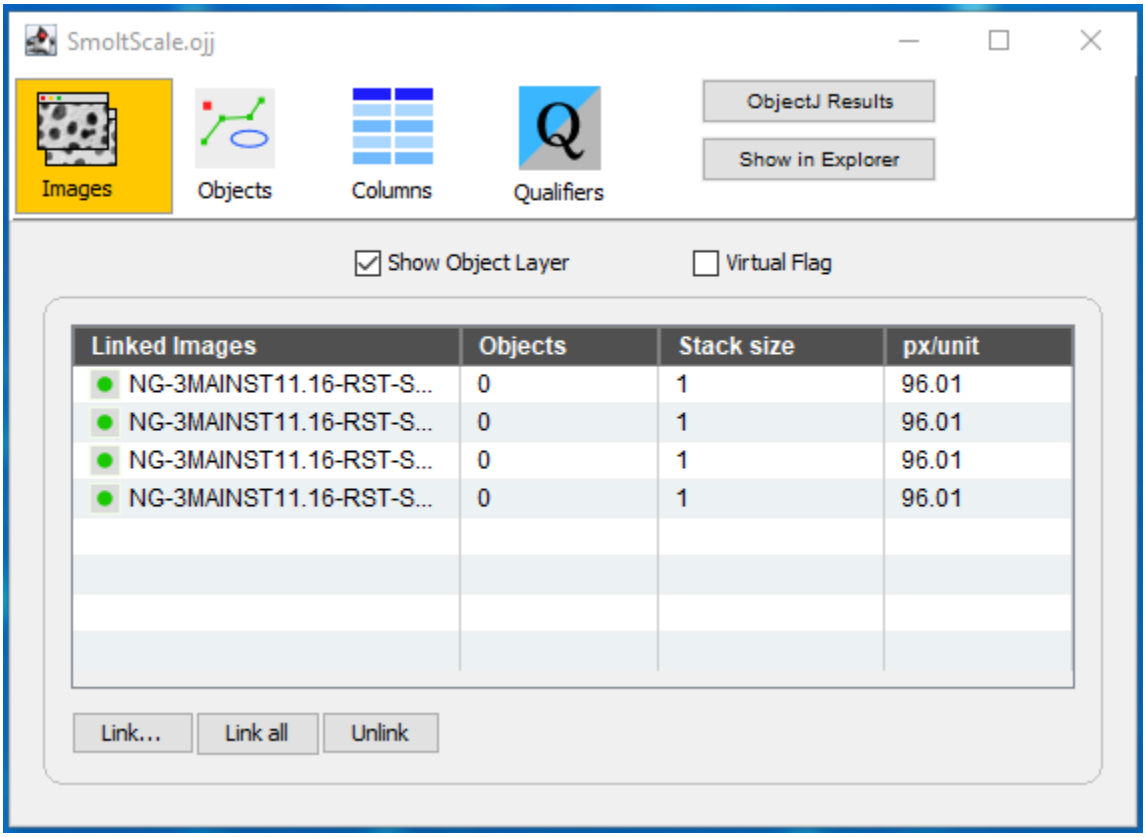
c. Each Column Title must be entered individually, which can be time consuming, but only necessary for the initial setup. The figure below stops at Circulus 5 (C5) for demonstration purposes.



d. Save project setup (**ImageJ** window > **ObjectJ** > **Save Project**)

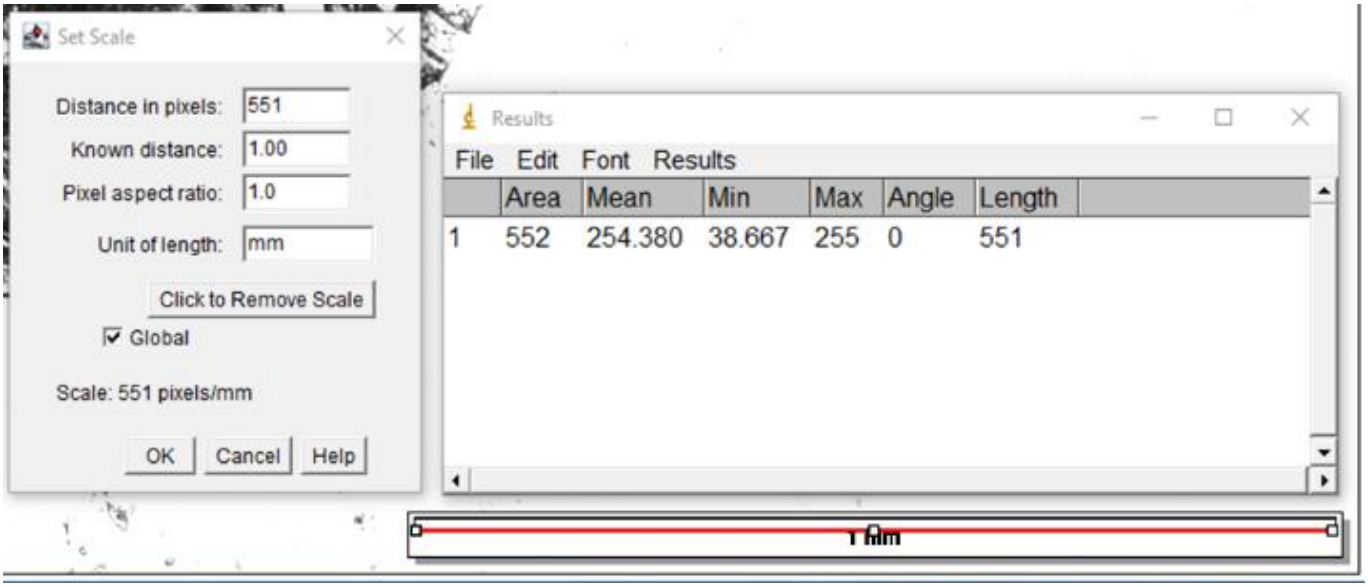
ImageJ transect measurements

1. Open ImageJ
2. Select **Plugins** > **jars** > **ObjectJ**
3. Object J will now appear to the left of plugins, select it then **Project** > **Open Project**
    a. Select the folder and the *.ojj project file you created during setup
4. To link images to your project, move images to be measured into your project folder.
    a. Select the Tree_Rings images (all three), then select **Unlink**. Delete these images from your project folder.
    b. Click **Link…** to link one image to your project or **Link all** to link all images in the project folder to your project. Linked images will appear in the window.

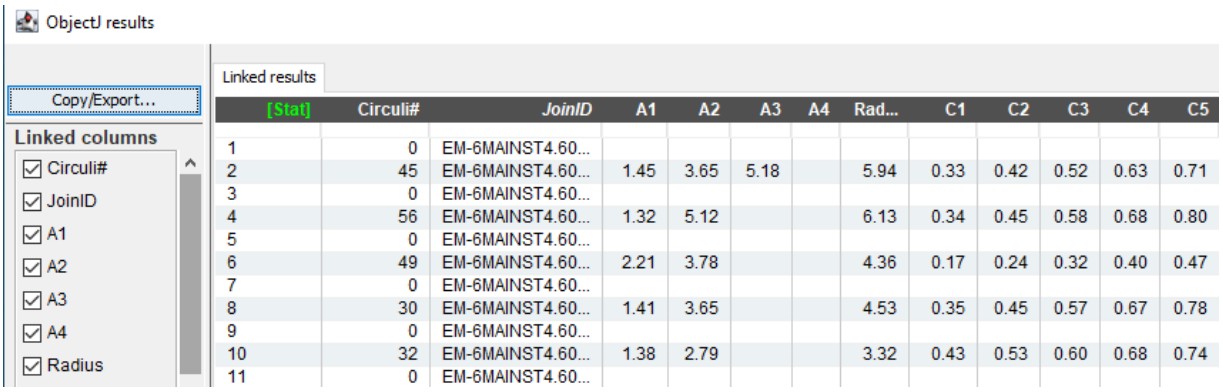


5. Double click on the first image to be measured. An image window will appear.
    a. In the ImageJ window, click **Analyze** > **Set Scale**, select **Click to Remove Scale**, then click **Ok**
    b. Select the **Line** tool  in the ImageJ window
    c. Draw a line the exact length of a scale bar on the image
    d. In the ImageJ window, click **Analyze** > **Measure**, and then the **Results** window will generate
    e. In the ImageJ Window, click **Analyze** > **Set Scale**
        i. In the **Set Scale** window, the **Distance in pixels** number should match the value in the *Length* column of the **Results** table window.

ii. Set the **Known distance** to the size of the scale bar
iii. Set the **Pixel aspect ratio** to 1.0
iv. Set **Unit of length** to mm
v. Check the **Global** box, then click **OK** to close **Set Scale** window



6. Use the **ObjectJ tools** window to select the **Transect scale feature** and draw the transect
    a. Adjust endpoint of transect by selecting **Move Tool**, then hold the **Alt** key while dragging endpoint to new location; select **Marker Tool** to return to marking features
7. Select the **Focus** feature and mark the start of the transect
8. Select each **scale feature** and mark it on the transect
    a. Remove a point using **Pistol** in ObjectJ tool
    b. Move point using the **Move Tool** with the **Alt** key
    c. Adding a point beyond the allowed columns but within max allowed clones will result in a new object in the results with no associated measurement data
9. Select **ObjectJ Results** in the project window and then click **Copy/Export**
    a. Two windows pop up, *Export ObjectJ* results and *Preview Text Output*
        i. In the *Export ObjectJ* results window, select **Include Headers** for export of first image; deselect for subsequent data
        ii. Deselect **Include Index Column**
        iii. Click **Copy**
    b. Paste the rows to an open Excel spreadsheet
    c. Rows with no data represent the transect as a separate object and should be deleted



[Stat]	Circuli#	JoinID	A1	A2	A3	A4	Rad...	C1	C2	C3	C4	C5
1	0	EM-6MAINST4.60...										
2	45	EM-6MAINST4.60...	1.45	3.65	5.18		5.94	0.33	0.42	0.52	0.63	0.71
3	0	EM-6MAINST4.60...										
4	56	EM-6MAINST4.60...	1.32	5.12			6.13	0.34	0.45	0.58	0.68	0.80
5	0	EM-6MAINST4.60...										
6	49	EM-6MAINST4.60...	2.21	3.78			4.36	0.17	0.24	0.32	0.40	0.47
7	0	EM-6MAINST4.60...										
8	30	EM-6MAINST4.60...	1.41	3.65			4.53	0.35	0.45	0.57	0.67	0.78
9	0	EM-6MAINST4.60...										
10	32	EM-6MAINST4.60...	1.38	2.79			3.32	0.43	0.53	0.60	0.68	0.74
11	0	EM-6MAINST4.60...										

**Image contains example ObjectJ results table for 5 scale measurements*

10. Use the *Snipping Tool* to save an overlay of the transect for auditing purposes; keep image open until overlay snip has been saved
11. Open next scale image
    a. If a message appears indicating a calibration error and suggests disabling **Global** setting, DO NOT disable or the *Calibration* will need resetting.
    b. DO check *Calibration*
        i. Select line tool and draw line on image scale bar
        ii. In the ImageJ window, click **Analyze** > **Measure**
        iii. Line length in *Results* window should reflect length of scale bar
    c. If *Calibration* is correct, continue by returning to Step 6.

QA/QC

PURPOSE: To provide consistency and repeatability for scale measurements, quality control, and quality assurance protocols have been developed.

1. First Reader measures all of the scales that are of adequate quality using image analysis software (e.g., Image-Pro 10) and enters the data in an Excel spreadsheet.
    a. Reader saves images with measurement overlays to an Overlay folder as .jpeg images with "SNAP" appended to the file name or something similar. This distinguishes the overlaid image from the original.

2. Second Reader audits 100% of ages and 30% (more or less depending on experience of first reader) of scale measurements using image overlays, and verifies correct placement of scale features.
    a. Second Reader remeasures scales if warranted.
    b. Second Reader identifies required edits to scale feature placements (i.e., annuli up or down one circulus etc.).
    c. The Auditor compares Readers' measurements to ensure precision.

3. The Auditor flags and reviews circulus values that indicate possible double marks or a missed circulus. The Auditor may automate measurement audits using an Excel macro to flag circulus values that are not within the 99% CI. Once the Auditor has identified outliers, they review the scale image, identify flag locations, and determine whether outliers are errors or true.

4. Once measurement audit is complete, the Auditor cross-references ages with the corresponding metadata using JoinIDs to identify and confirm tagged or marked fish (known age). Auditors revise with the known-age information.

SCALE READING PROCEDURES

Origin determination

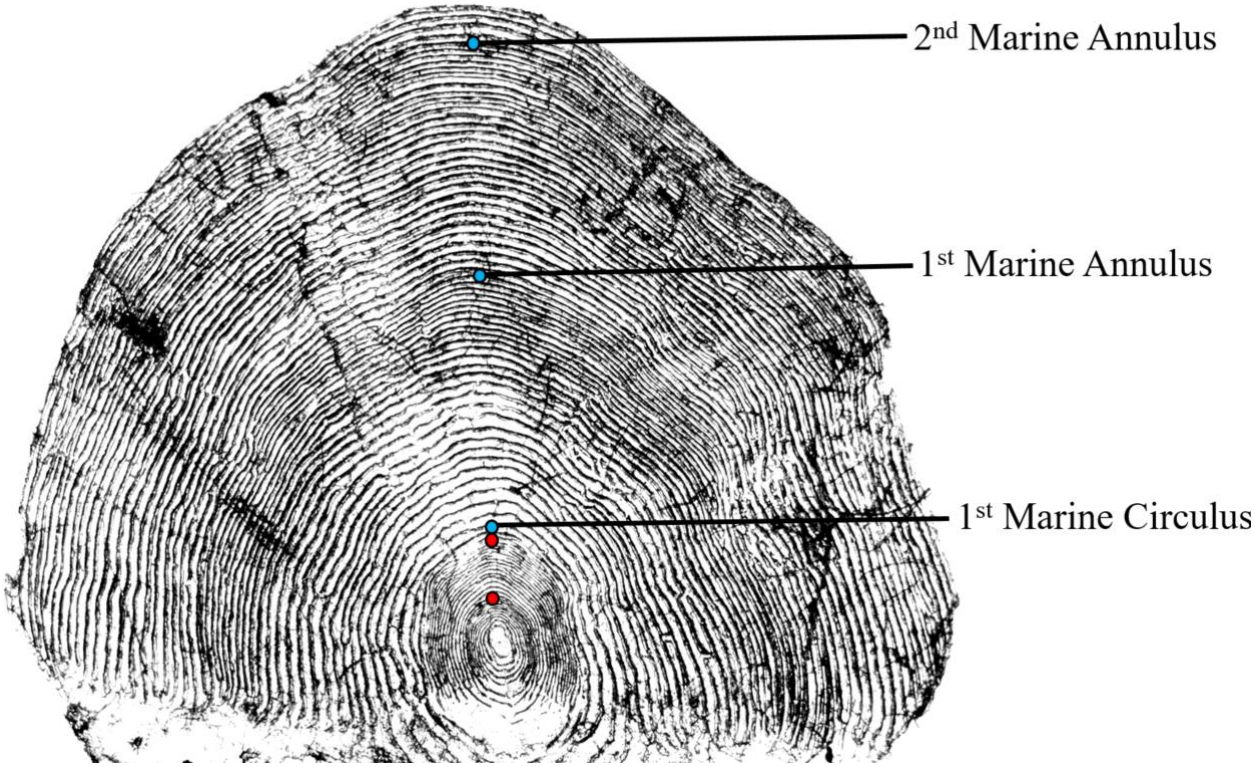
Scale-based origin determination methods are based on ICES (1992) and have been utilized to discriminate between wild (naturally reared) salmon and hatchery salmon

Age Readers identify hatchery origin scales by identifying uniform circuli spacing that reflects growth occurring in hatchery conditions where environmental conditions and feeding patterns are more consistent than in a river.

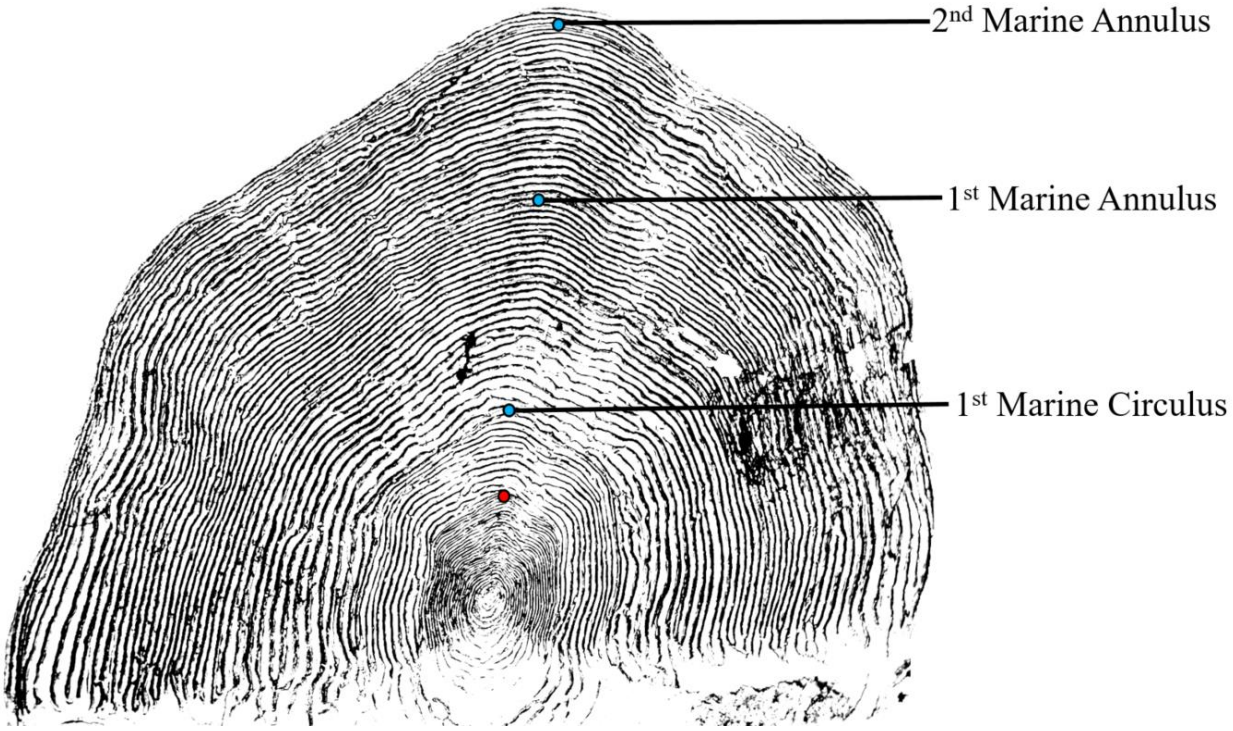
Smolt scales

The scale on the left is from naturally reared age-2 smolt (w.2). The scale on the right is from a parr-stocked age-2 smolt (p20). Note the reduced variation in circuli spacing for hatchery scale.

Adult scales



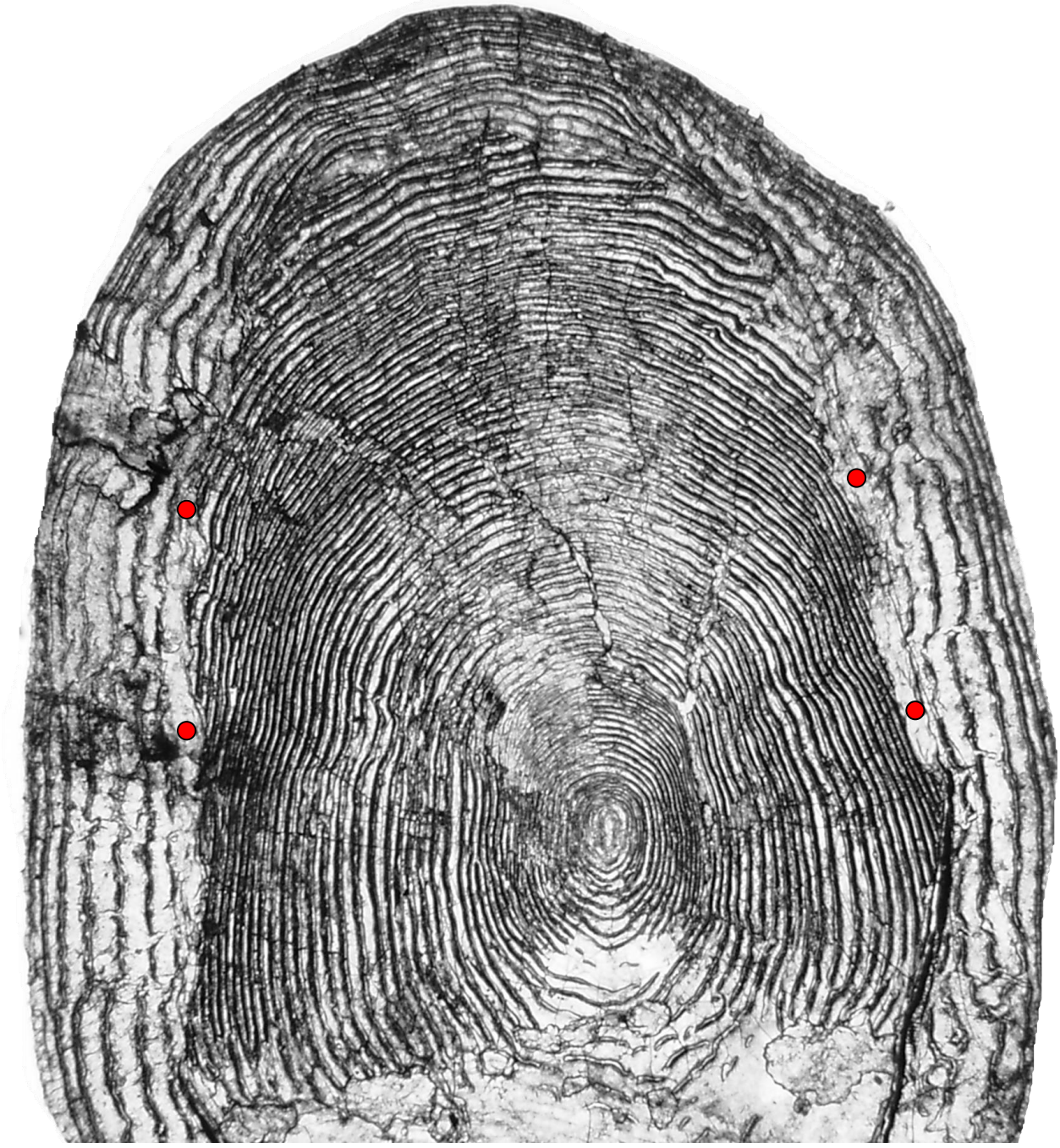
Scale from two sea-winter adult salmon sampled 17 June 1989 from the Penobscot river while returning to spawn. Aged as 2.2. Freshwater annuli are marked in red. Marine annuli are marked in blue. This scale was determined to be naturally reared or of wild origin due to the two distinct freshwater annuli with clear separation of seasonal growth.



Scale from two sea-winter adult salmon sampled 25 May 2002 on the Penobscot river while returning to spawn. Freshwater annuli are marked in green. Aged as 1.2. Freshwater annuli are marked in red. Marine annuli and the first marine circulus are marked in blue. This scale was determined to be of hatchery origin due to the uniform circulus spacing in the freshwater zone.

Spawning marks

Atlantic salmon scales provide a record of spawning because of erosion that occurs on the scales during the spawning migration and freshwater spawning period.



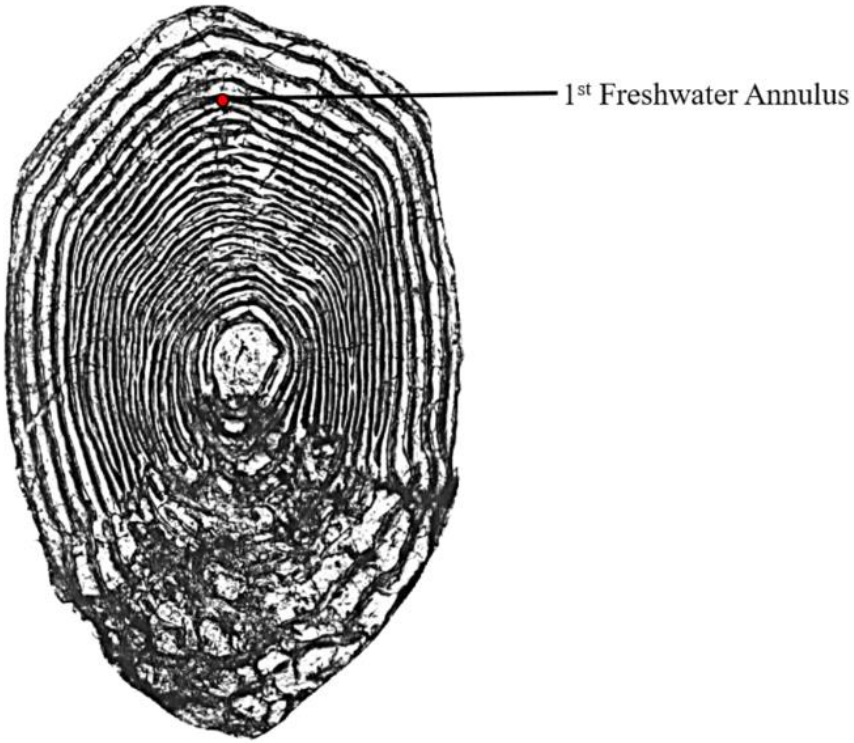
Scale from two sea-winter adult salmon sampled in 1967 on the Penobscot River with spawning scars marked in red.

Aging

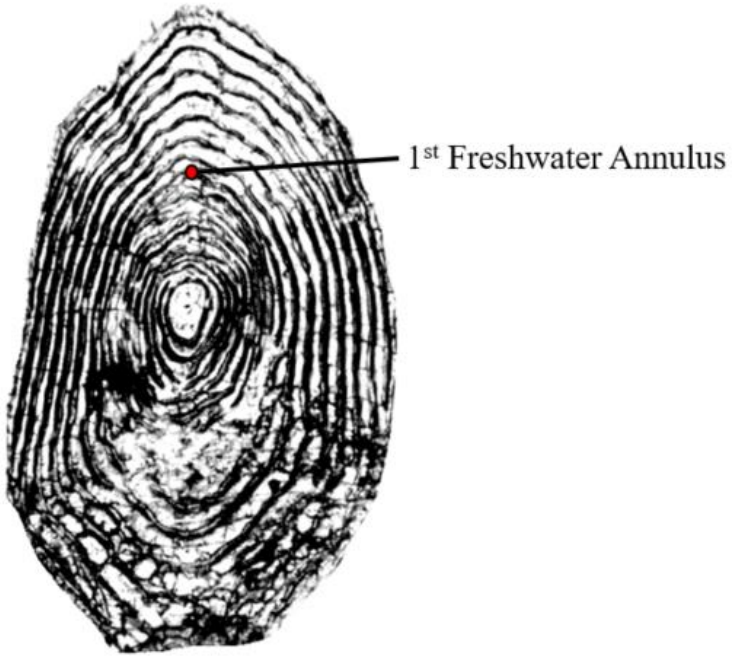
Aging methods are based on ICES (1992) where an annulus is identified by either crossing over or by increasing circulus spacing following the winter band.

Age 1 naturally reared smolts

Scale from age 1 naturally reared (w.1) smolt sampled 27 May 2011 on the Narraguagus River



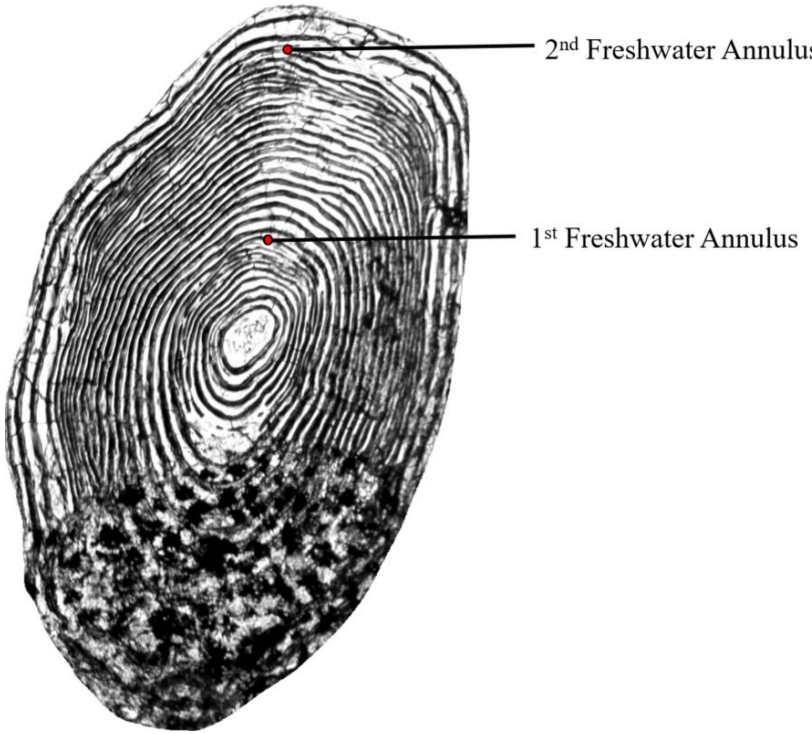
Scale from age 1 naturally reared (w.1) smolt sampled 18 May 2022 on the Sandy River



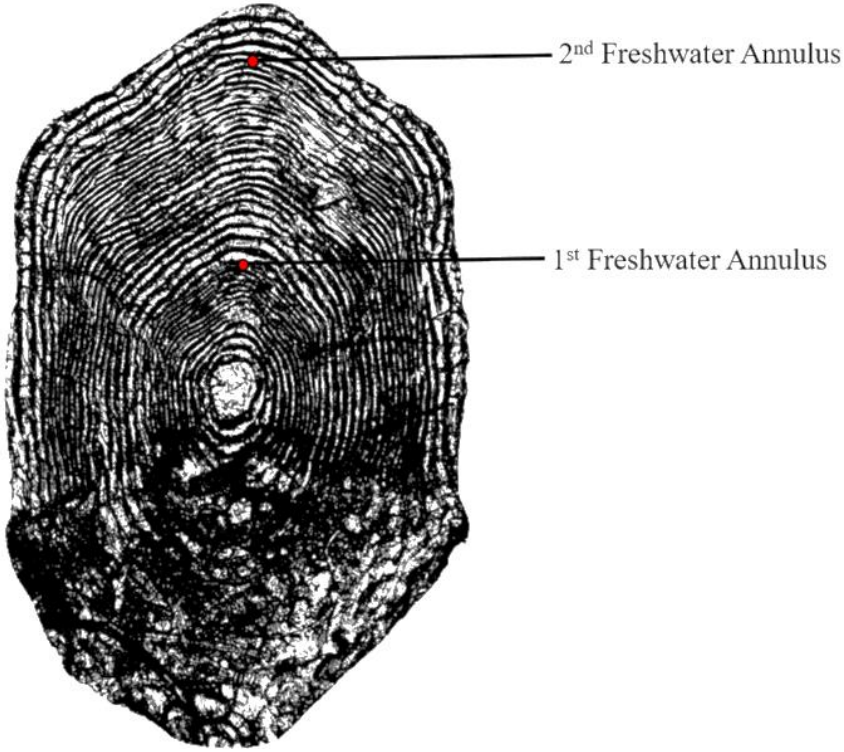
Scale from age 1 naturally reared (w.1) smolt sampled 29 May 2011 on the Sheepscot River

Scale from an age 1 naturally reared (w.1) smolt sampled 9 May 2011 on the Sheepscot River

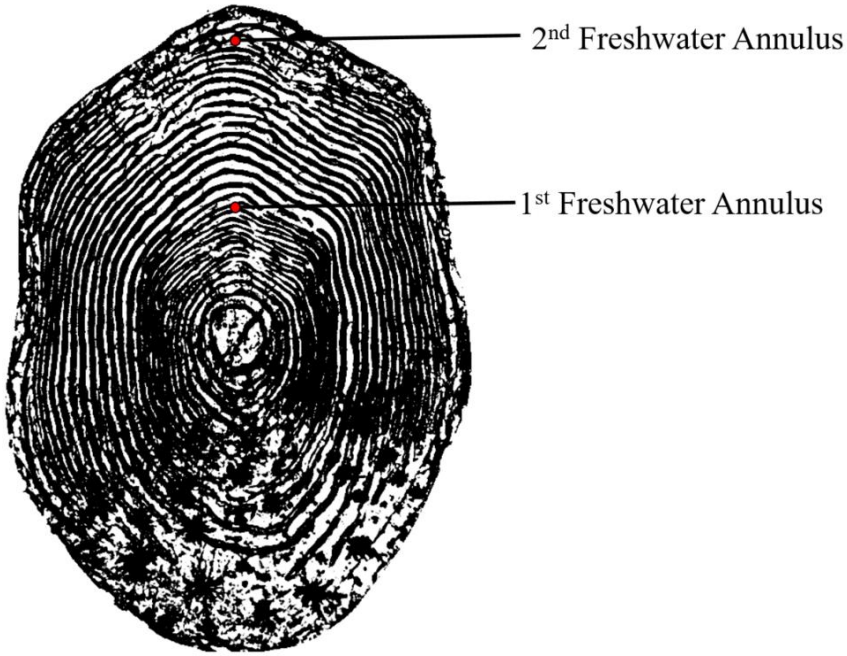
Age 2 naturally reared smolts



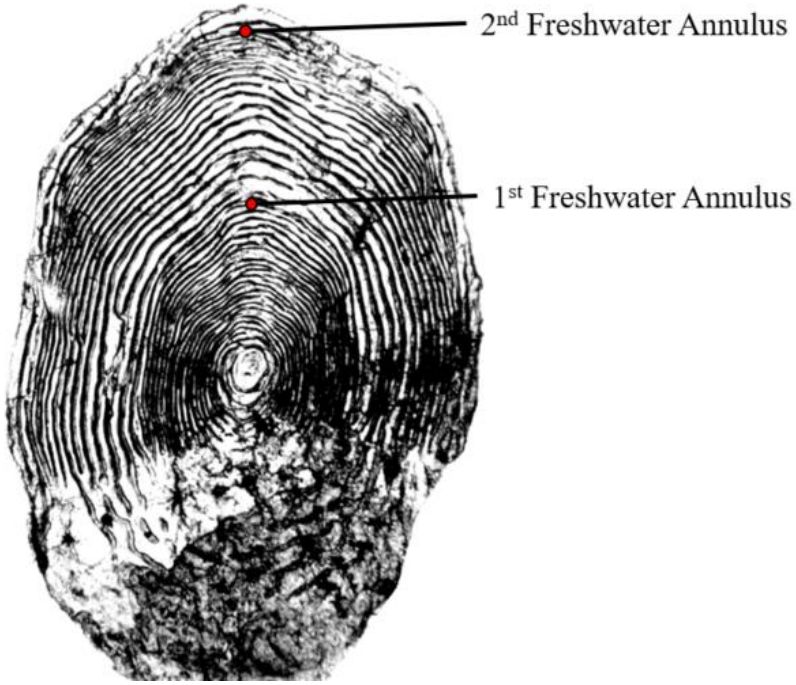
Scale from naturally reared age 2 smolt (w.2) sampled 29 April 2006 on the Narraguagus River



Scale from naturally reared age 2 smolt (w.2) sampled 21 May 2021 on the Sandy River

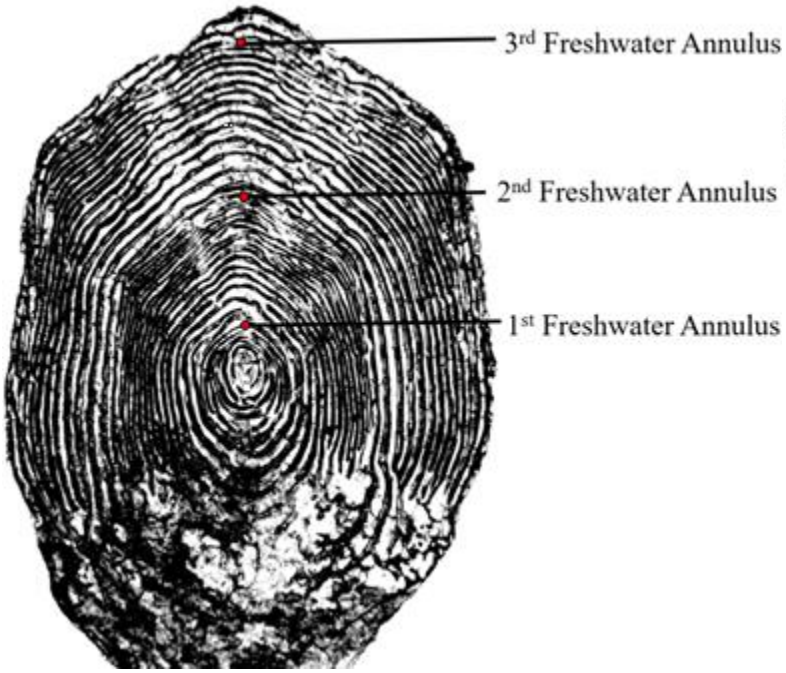


Scale from naturally reared age 2 smolt (w.2) sampled 16 May 2015 on the Piscataquis River

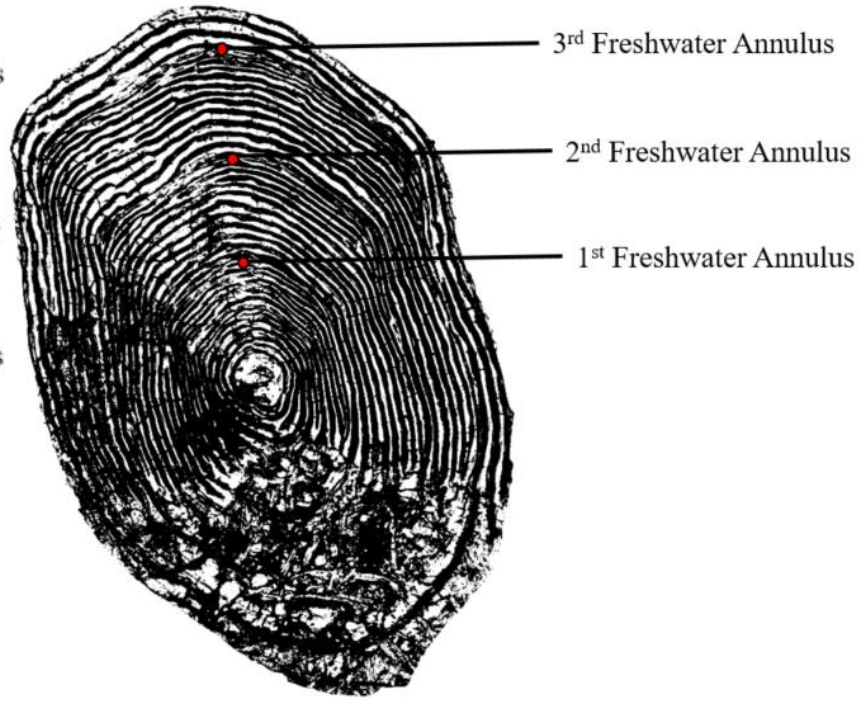


Scale from naturally reared age 2 smolt (w.2) sampled 3 May 2011 on the Sheepscot River

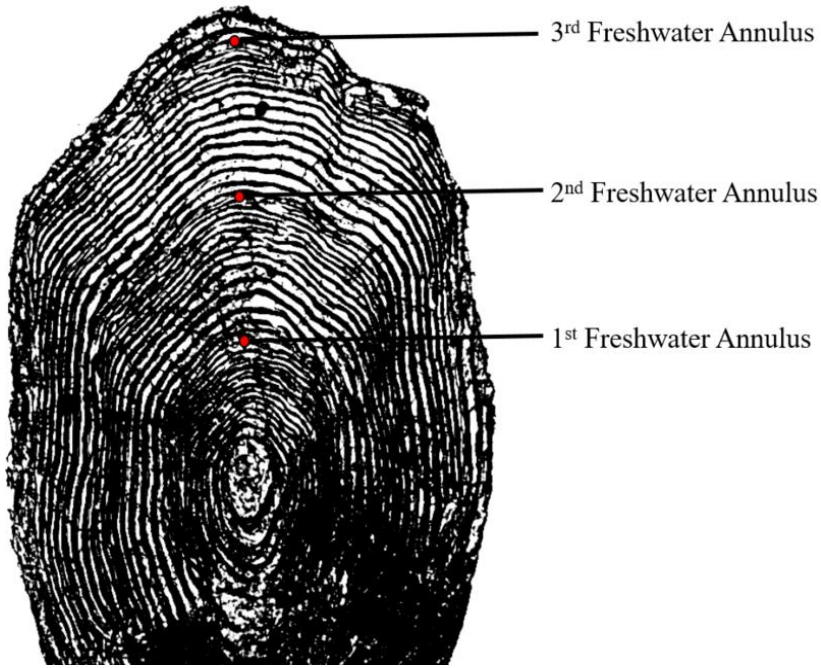
Age 3 naturally reared smolts



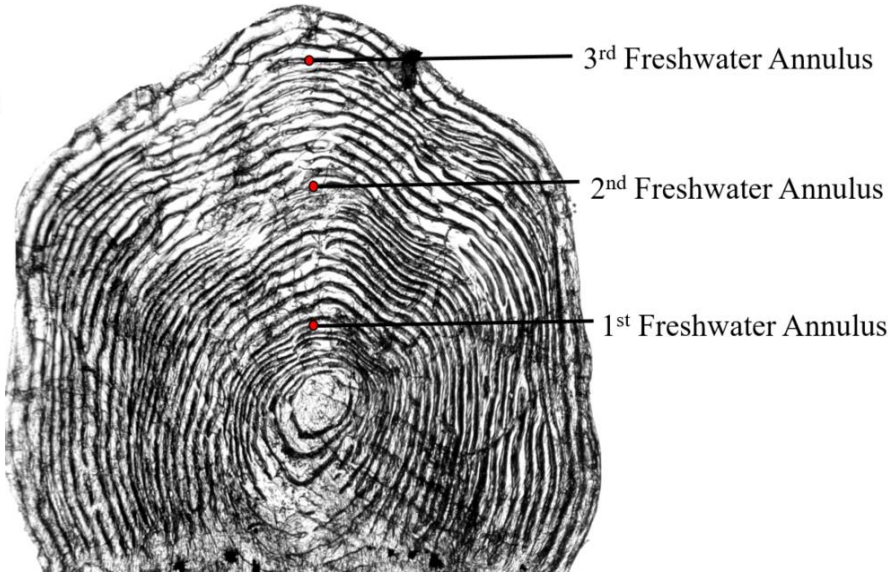
Scale from naturally reared age 3 smolt (w.3) sampled 20 May 2011 on the Sheepscot River



Scale from naturally reared age 3 smolt (w.3) sampled 21 May 2021 on the Sandy River

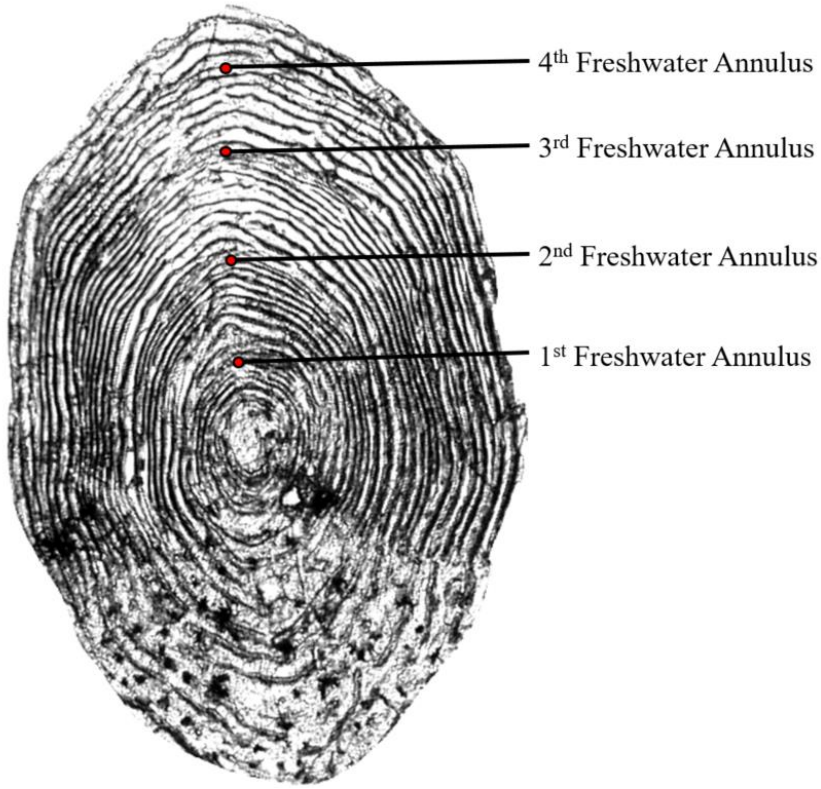


Scale from naturally reared age 3 smolt (w.3)
sampled 15 May 2015 on the Piscataquis River

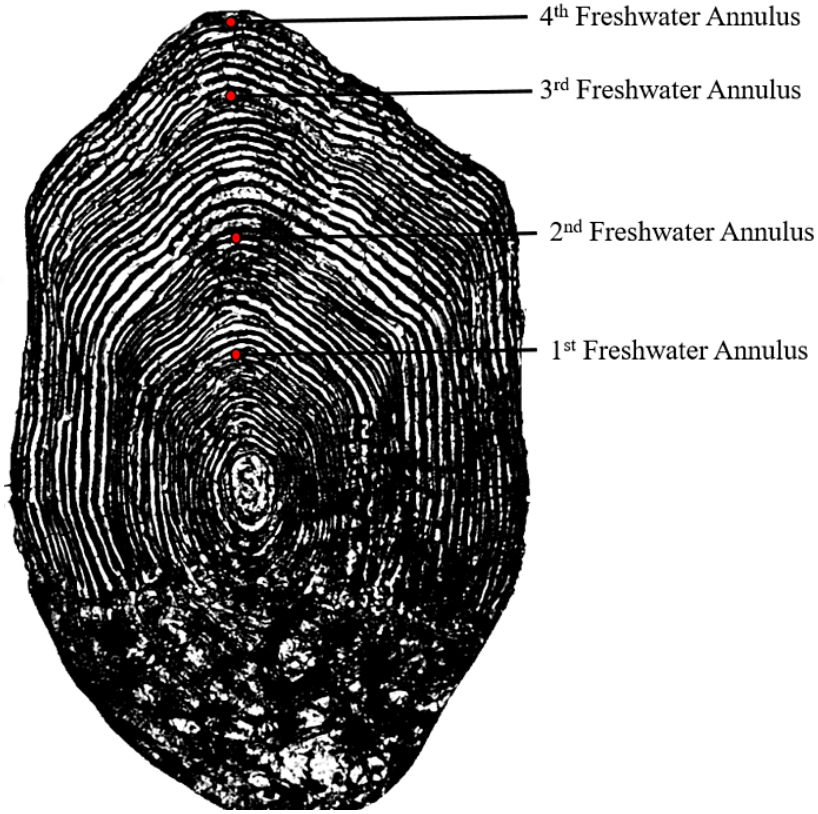


Scale from naturally reared age 3 smolt (w.3)
sampled 30 April 2006 on the Narraguagus River

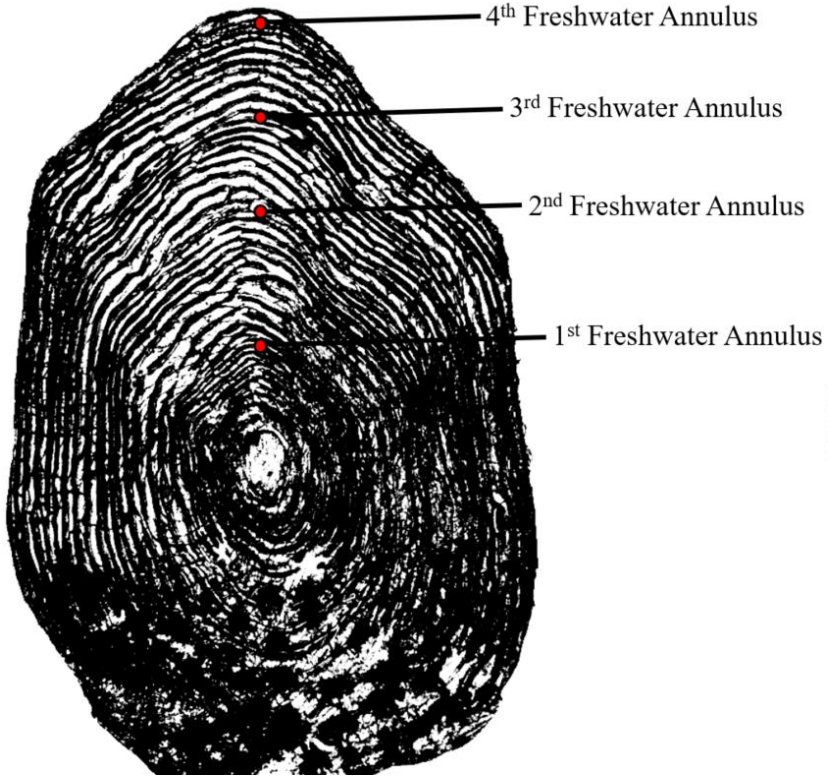
Age 4 naturally reared smolts



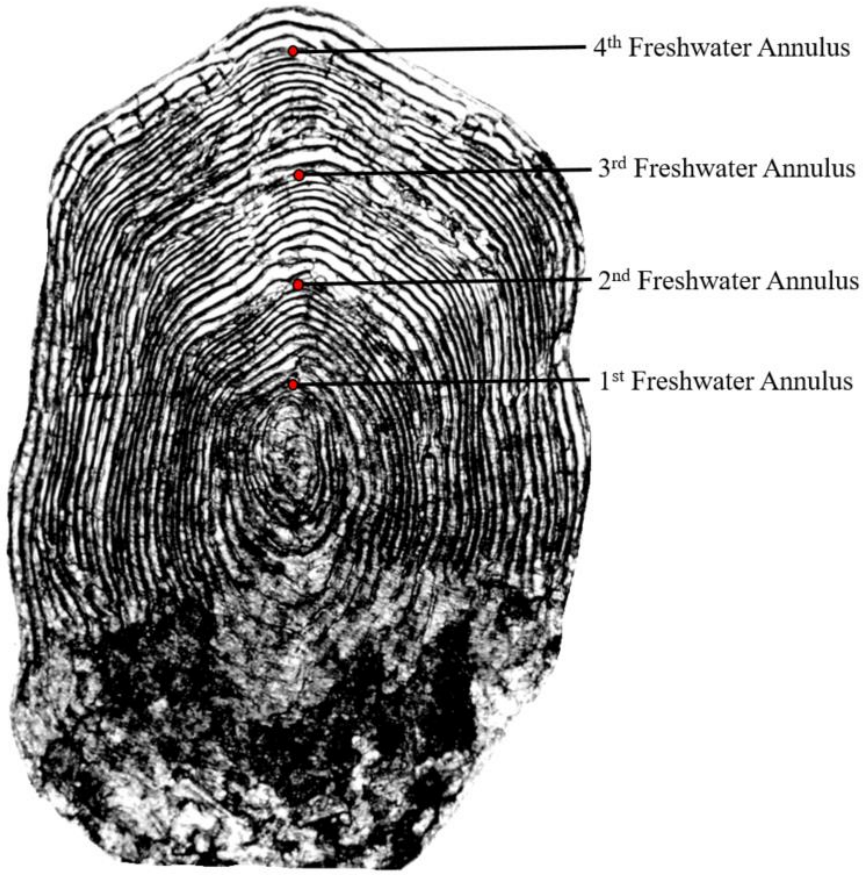
Scale from naturally reared age 4 smolt (w.4) sampled 24 May 2000 on the Narraguagus River



Scale from naturally reared age 4 smolt (w.4) sampled 22 April 2012 on the Piscataquis River

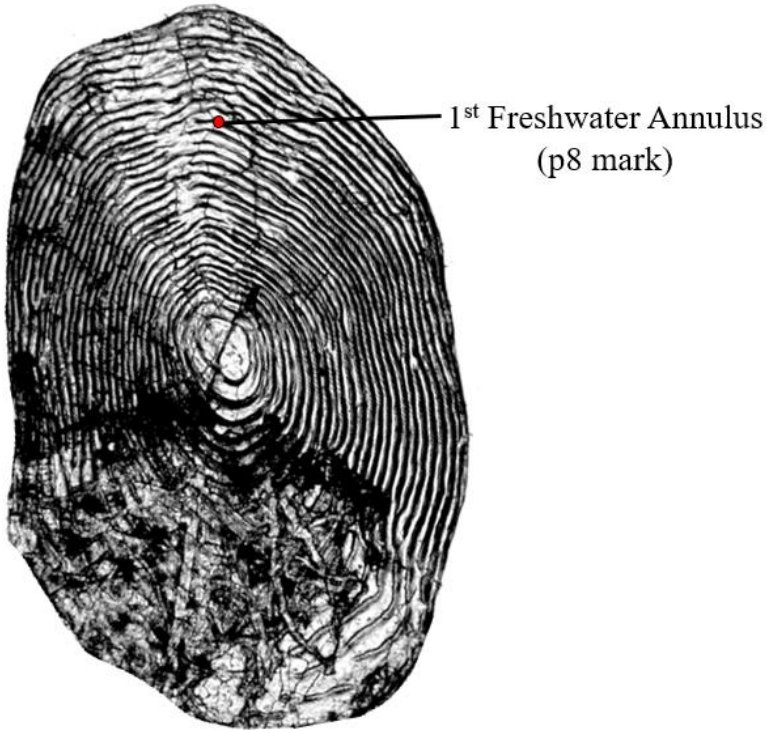


Scale from naturally reared age 4 smolt (w.4) sampled 17 May 2021 on the Sandy River

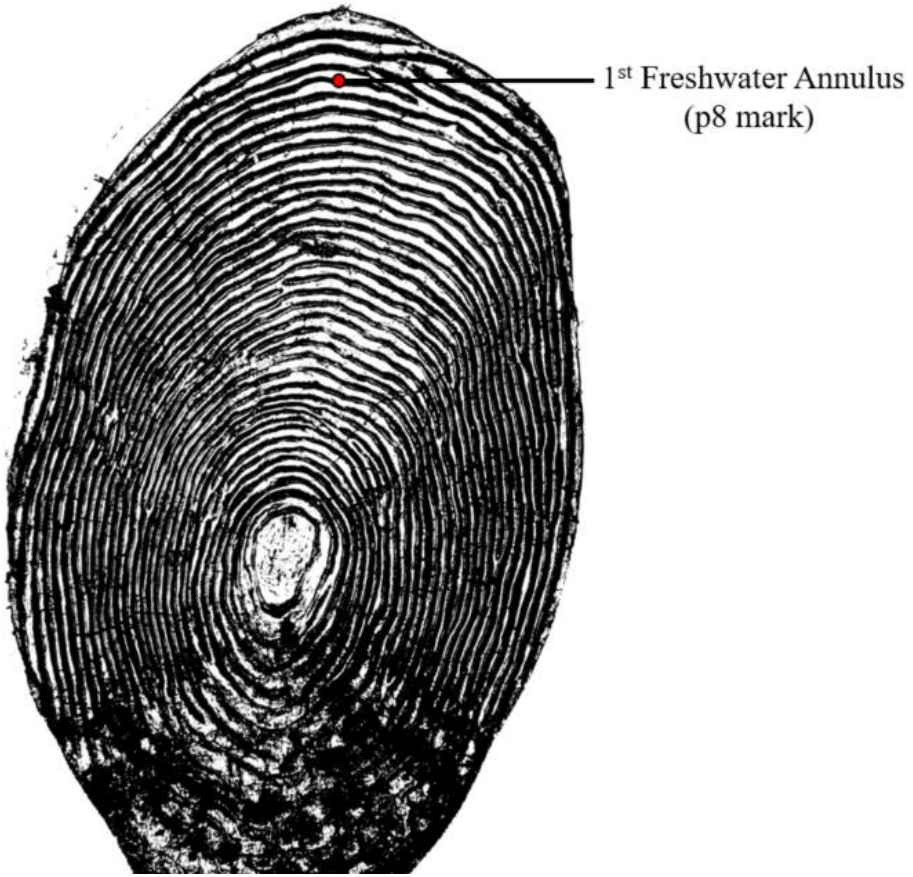


Scale from naturally reared age 4 smolt (w.4) sampled 7 May 2011 on the Piscataquis River

Age 1 (p8) parr-stocked smolts

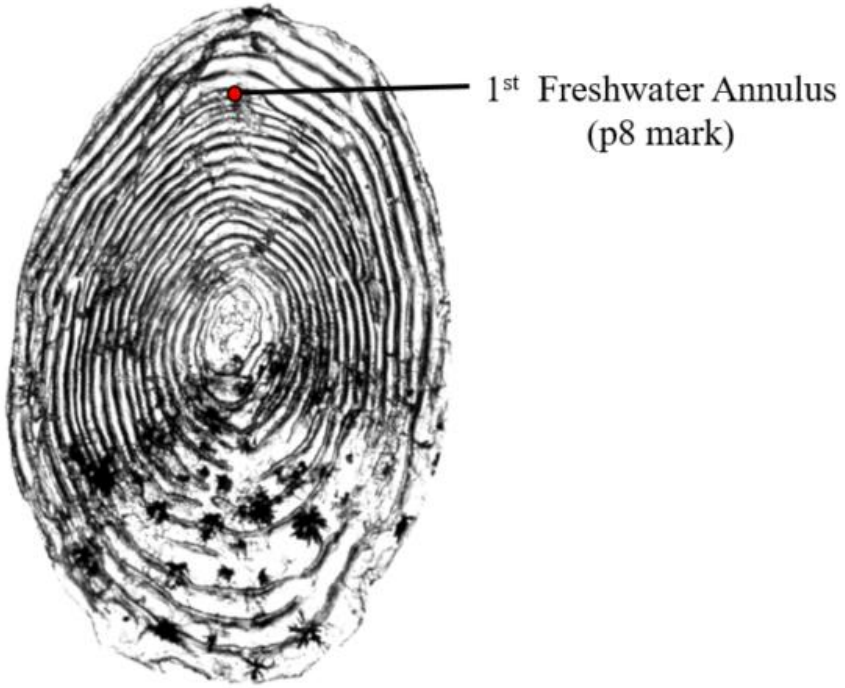


Scale from parr-stocked age 1 smolt (p8) sampled 9 May 2004 on the Dennys River



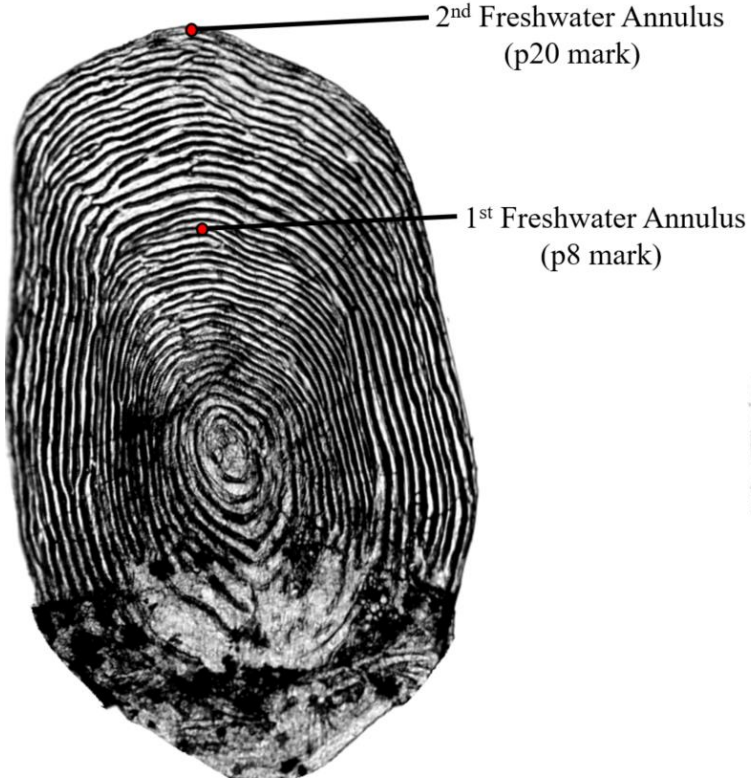
Scale from parr-stocked age 1 smolt (p8) sampled 7 May 2014 on the East Machias River

Scale from parr-stocked age 1 smolt (p8)
sampled 20 May 2005 on the Sheepscot River

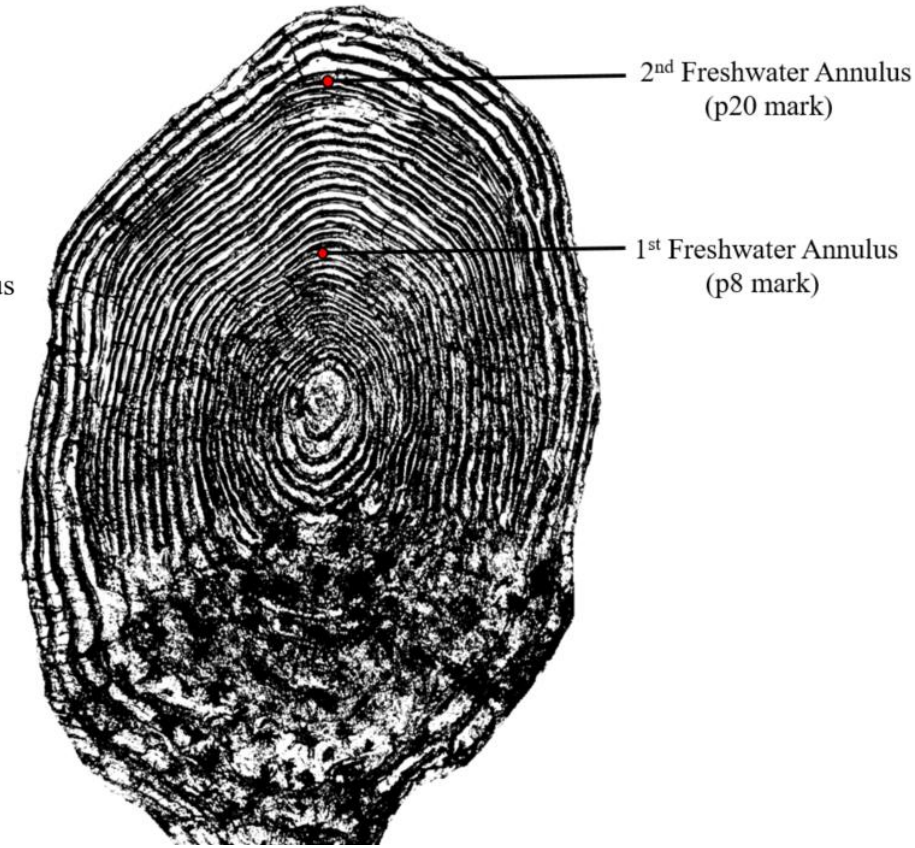


Scale from parr-stocked age 1 smolt (p8)
sampled 20 May 2005 on the Sheepscot River

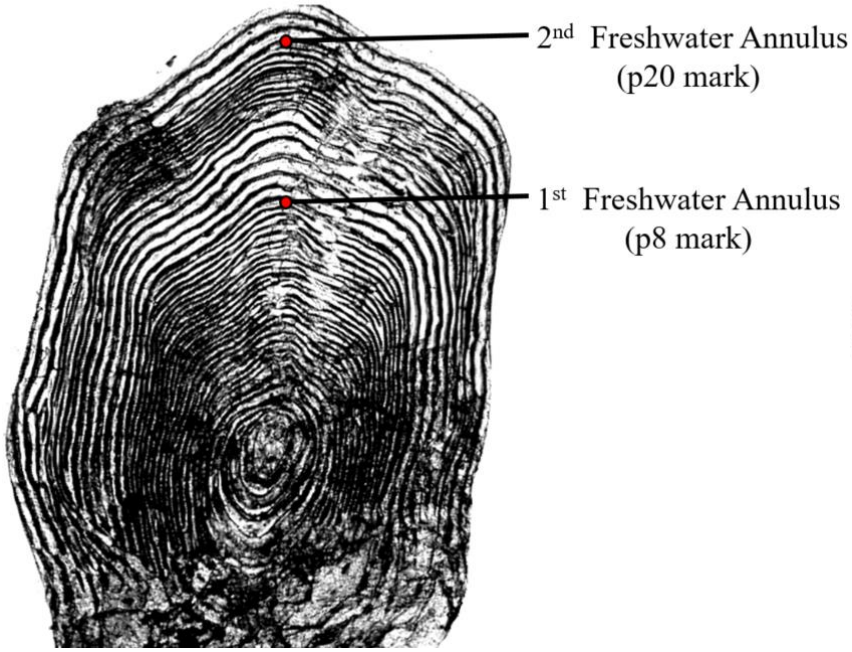
Age 2 (p20) parr-stocked smolts



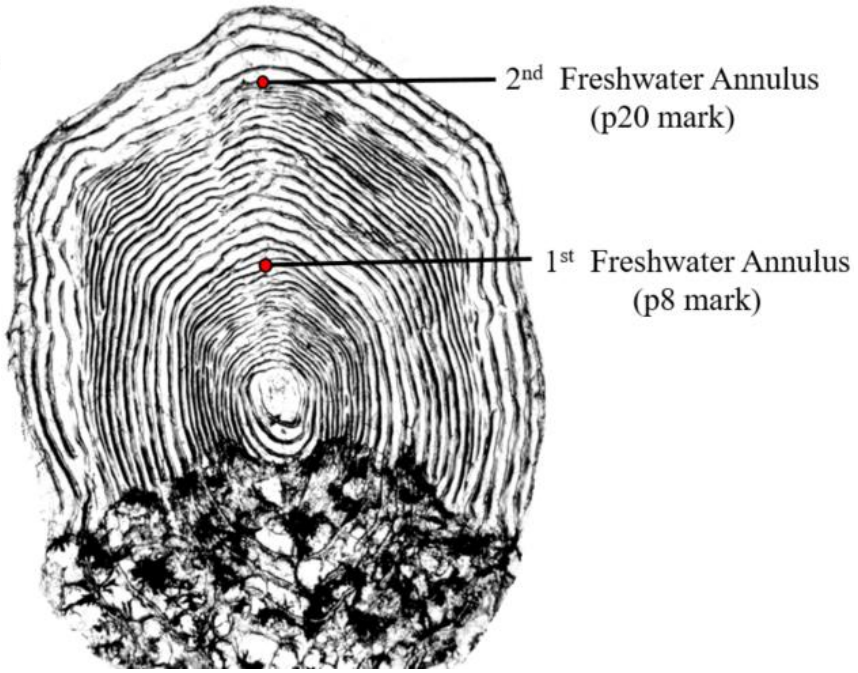
Scale from parr-stocked age 2 smolt (p20) sampled 5 May 2004 on the Dennys River



Scale from parr-stocked age 2 smolt (p20) sampled 19 May 2015 on the East Machias River

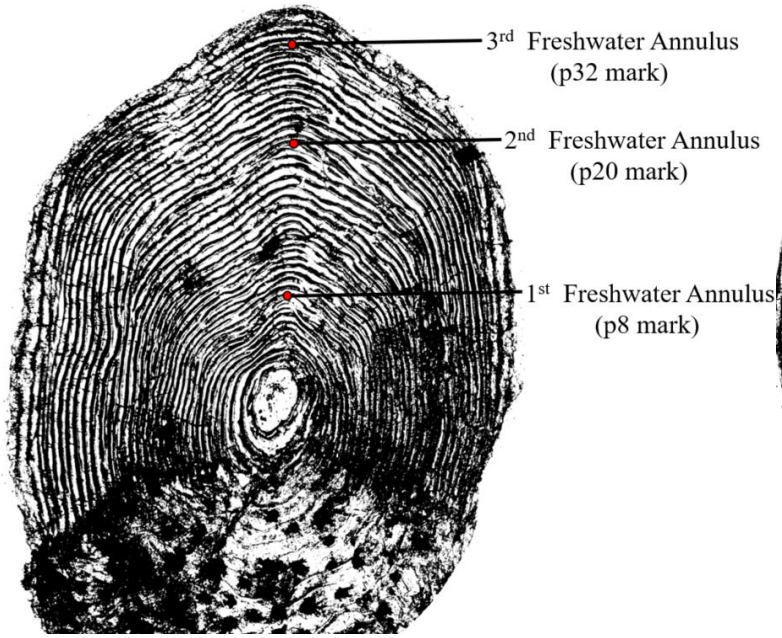


Scale from parr-stocked age 2 smolt (p20)
sampled 11 May 2000 on the Narraguagus River

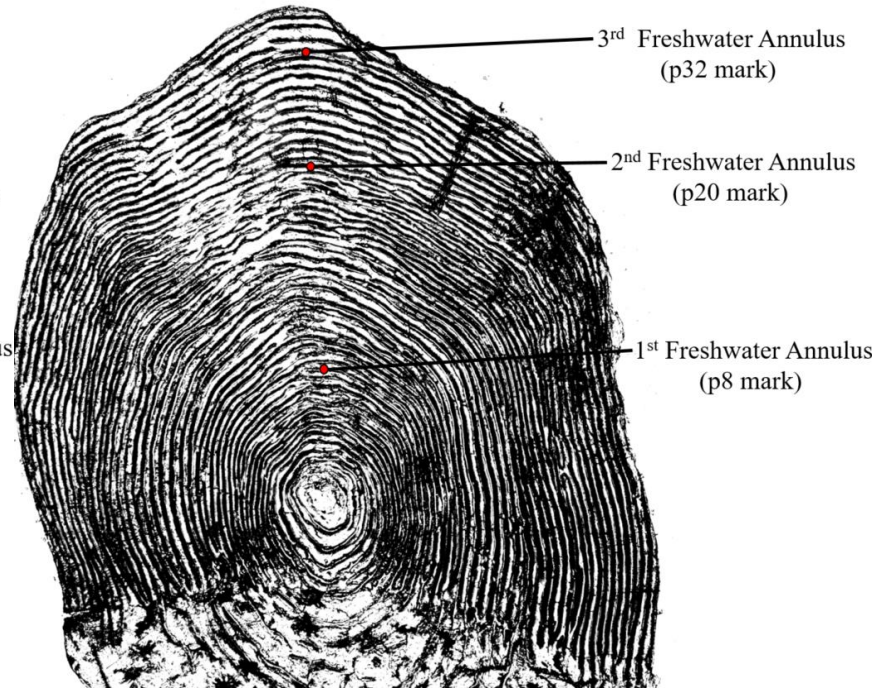


Scale from parr-stocked age 2 smolt (p20)
sampled 16 May 2014 on the Sheepscot River

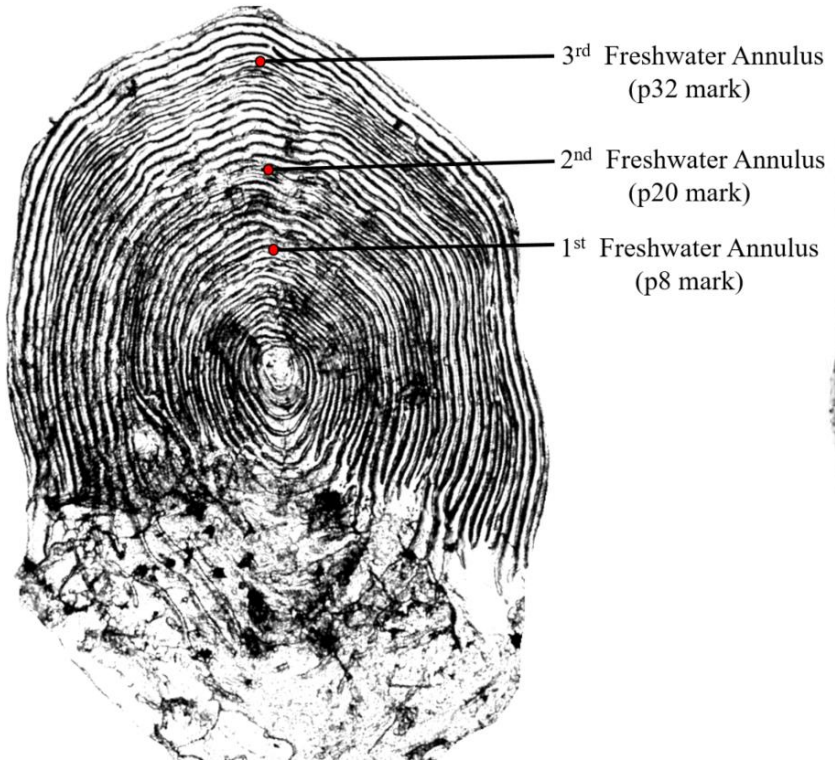
Age 3 (p32) parr-stocked smolts



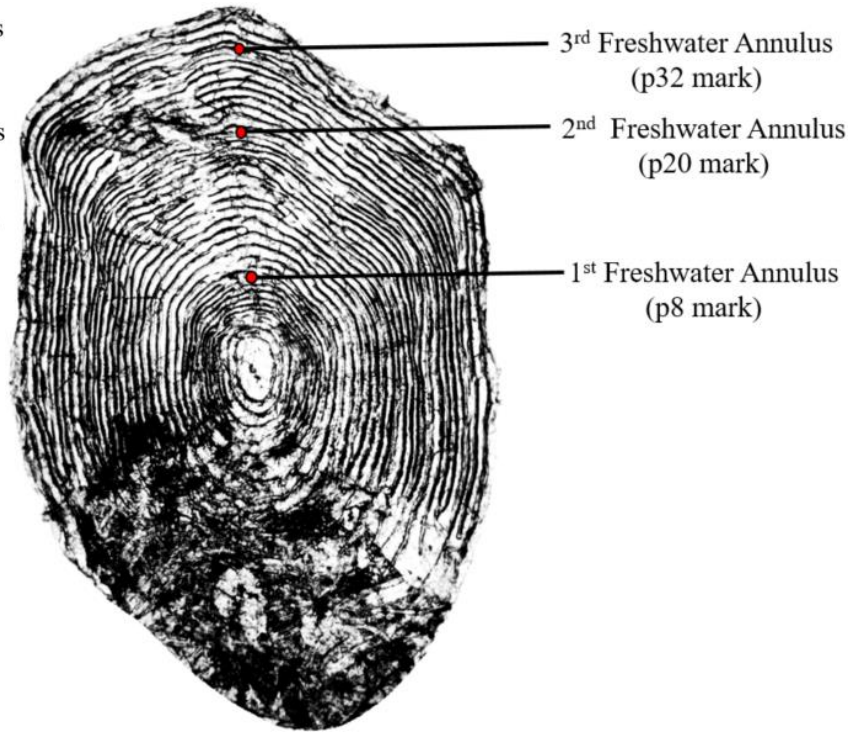
Scale from parr-stocked age 3 smolt (p32)
sampled 7 May 2015 on the East Machias River



Scale from parr-stocked age 3 smolt (p32)
sampled 24 May 2014 on the East Machias River

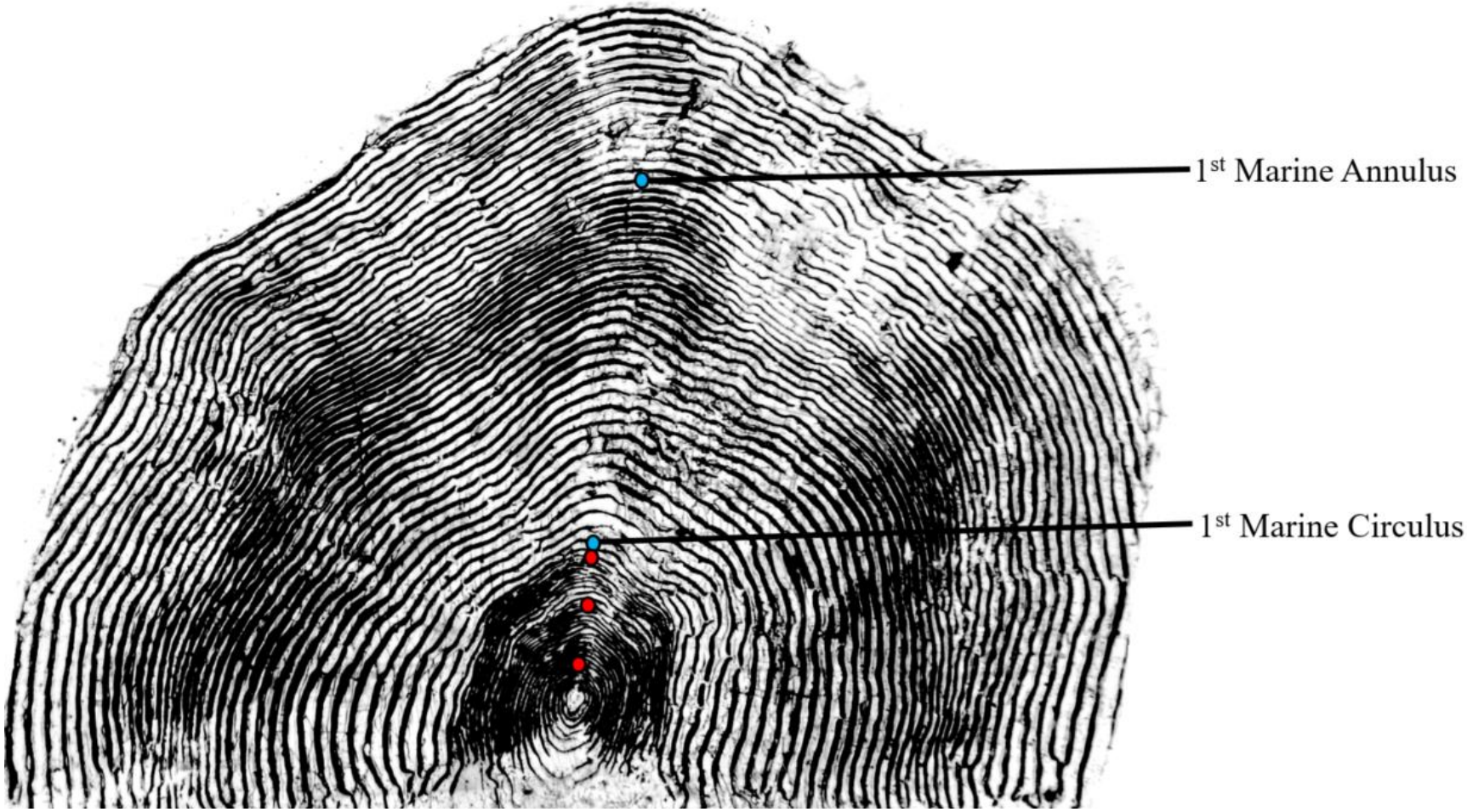


Scale from parr-stocked age 3 smolt (p32)
sampled 12 May 2010 on the Narraguagus River

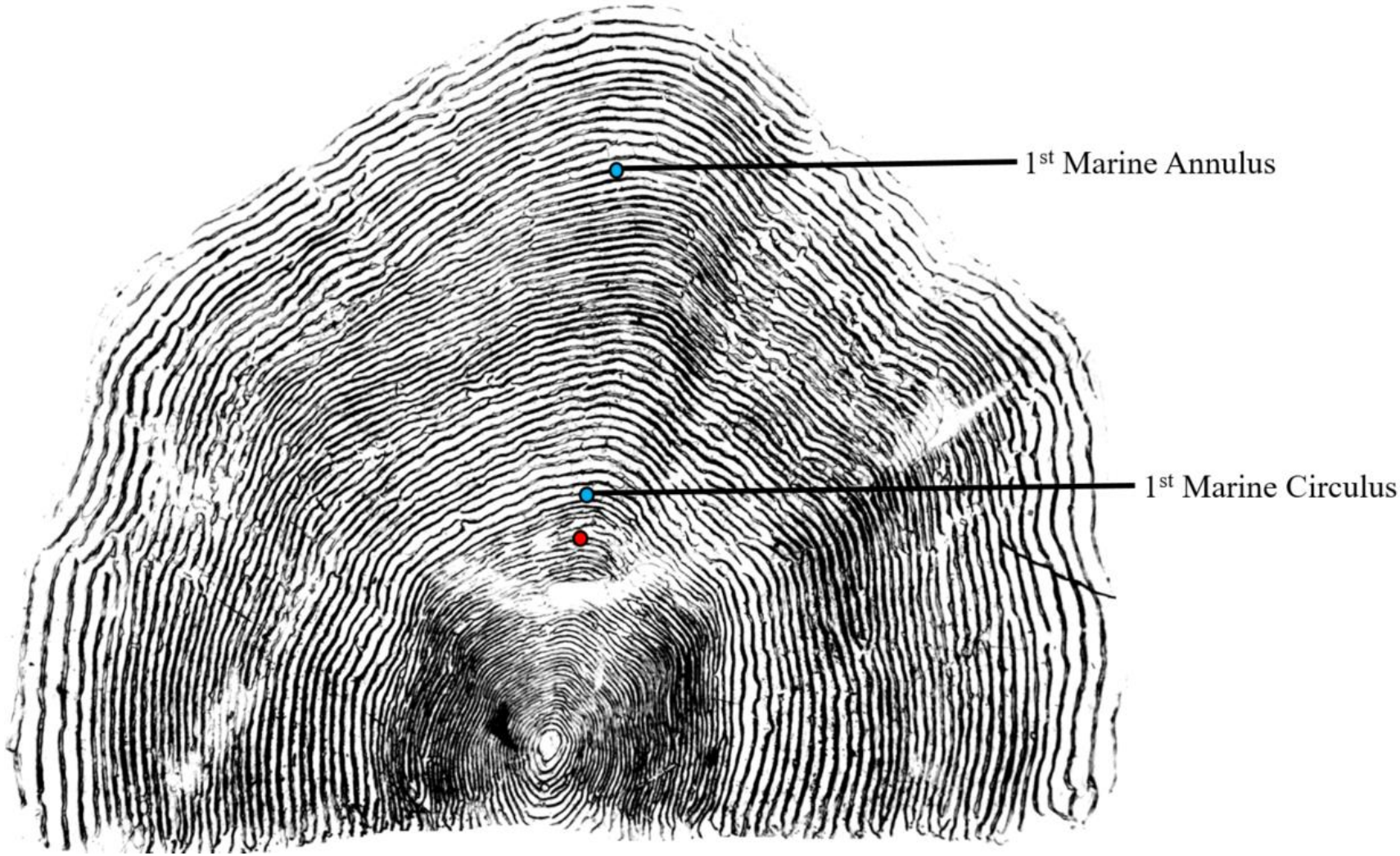


Scale from parr-stocked age 3 smolt (p32)
sampled 16 May 2014 on the Sheepscot River

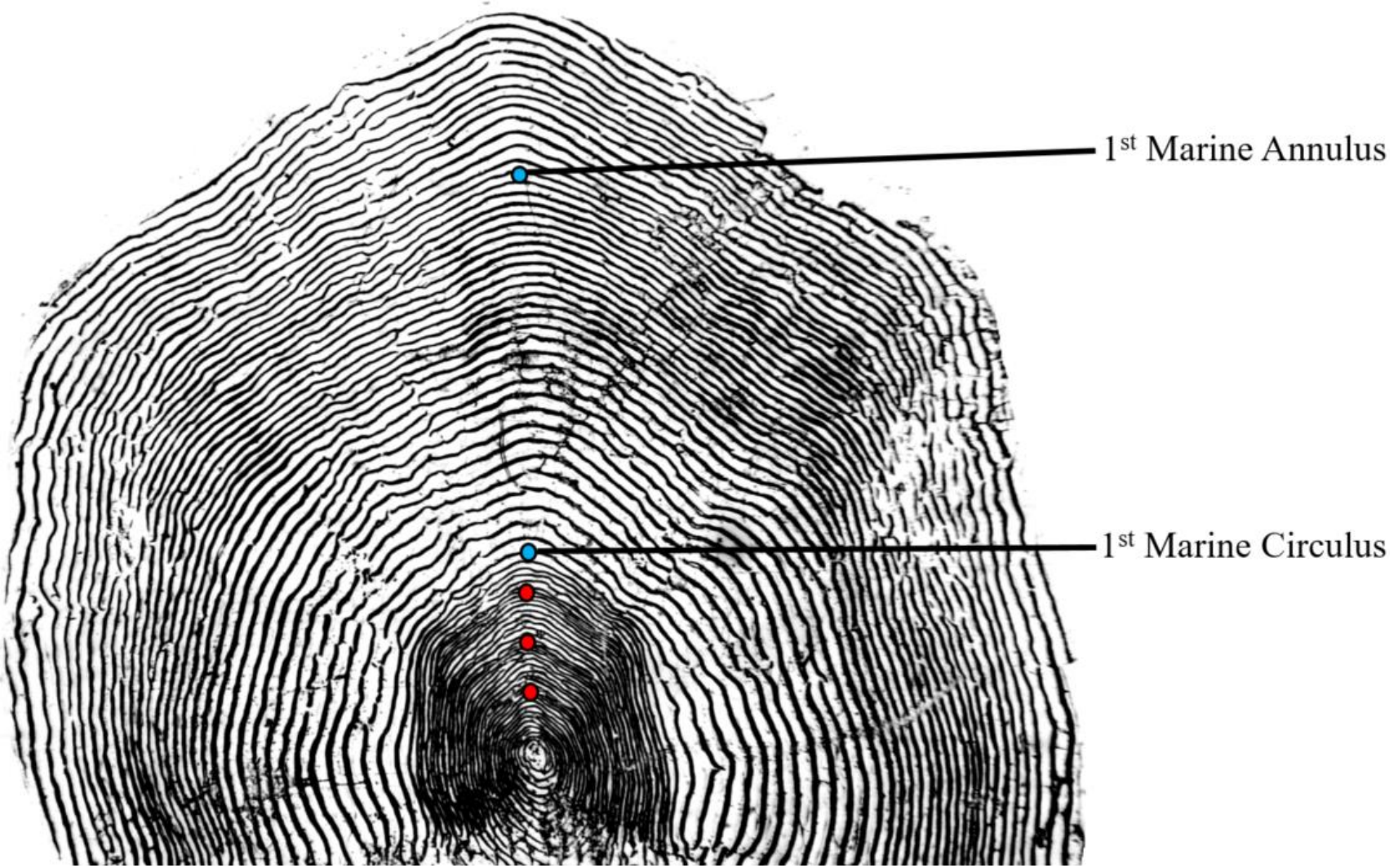
1SW Non-maturing Adults



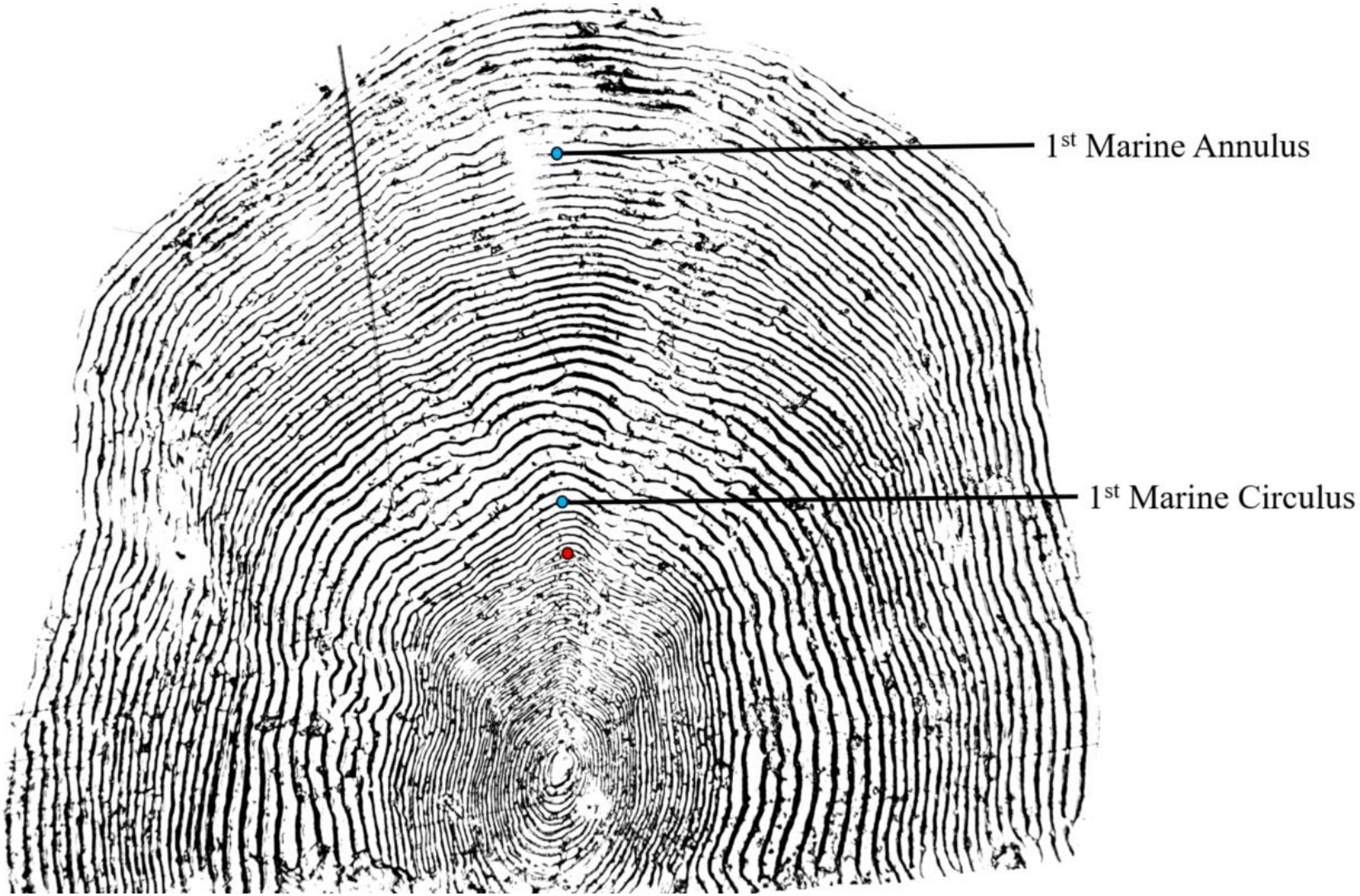
Scale from adult salmon sampled 15 September 2016 in Sisimuit, West Greenland. Freshwater annuli are marked in red. Region of origin confirmed as United States from fin clip genetics.



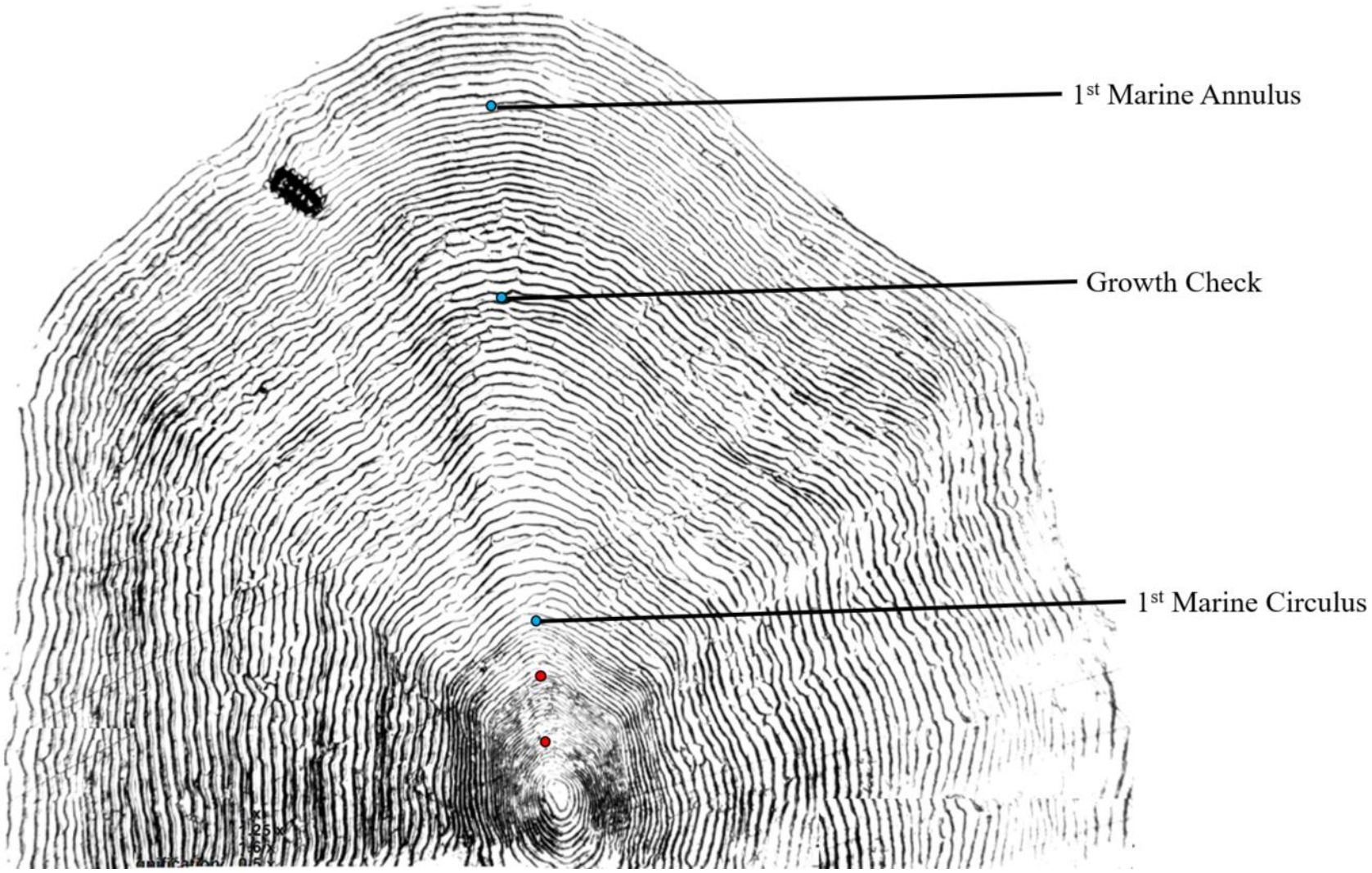
Scale from adult salmon sampled 21 September 2011 in Sisimiut, West Greenland. Freshwater annuli are marked in red. Region of origin confirmed as United States from fin clip genetics.



Scale from adult salmon sampled 12 September 2018 in Manisoq, West Greenland. Freshwater annuli are marked in red. Region of origin confirmed as United States from fin clip genetics.

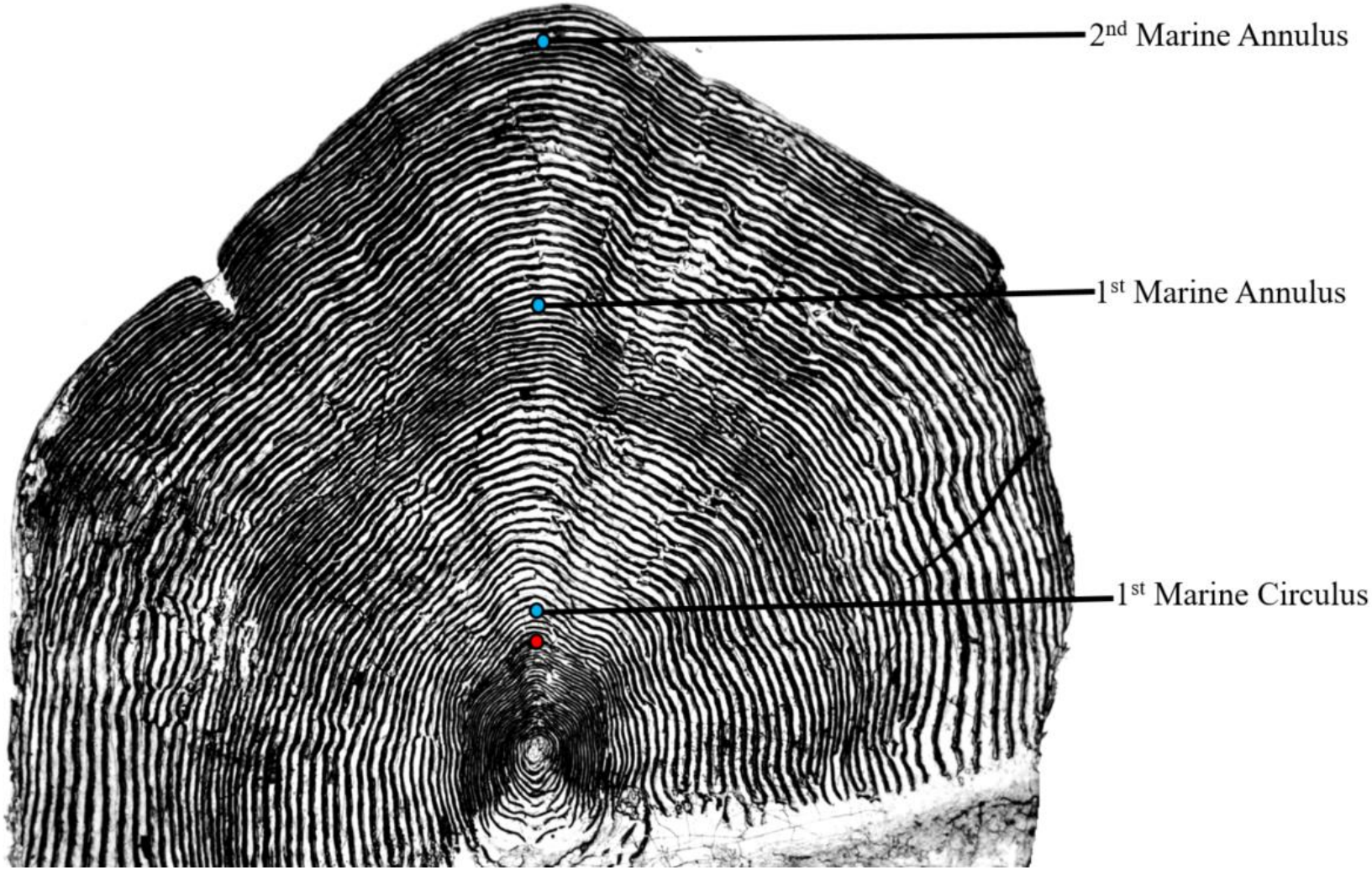


Scale from adult salmon sampled 17 August 2022 in Paamuit, West Greenland. Freshwater annuli are marked in red. Region of origin confirmed as United States from fin clip genetics.

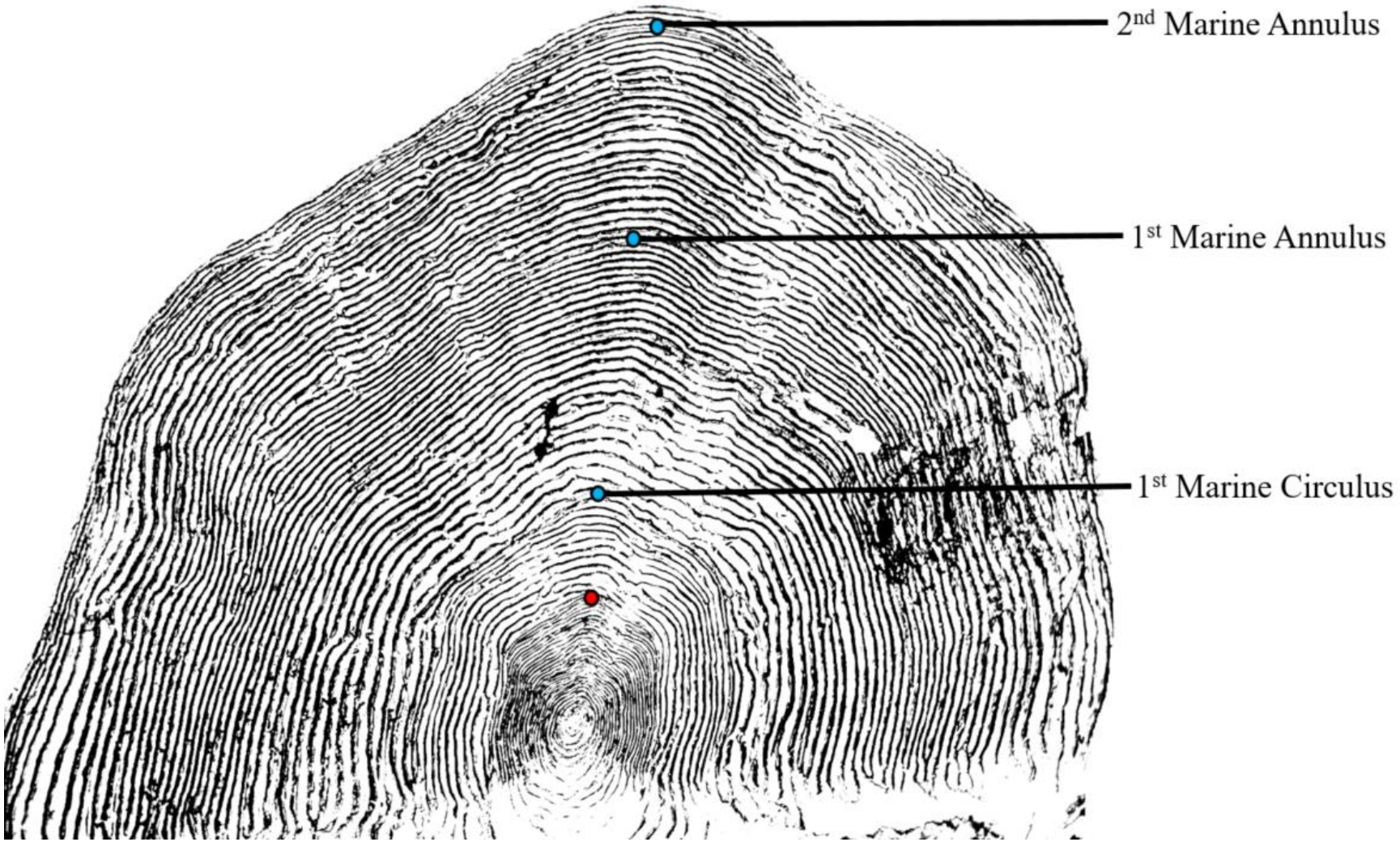


Scale from adult salmon sampled 17 August 2022 in Paamuit, West Greenland. Freshwater annuli are marked in red. Region of origin confirmed as United States from fin clip genetics.

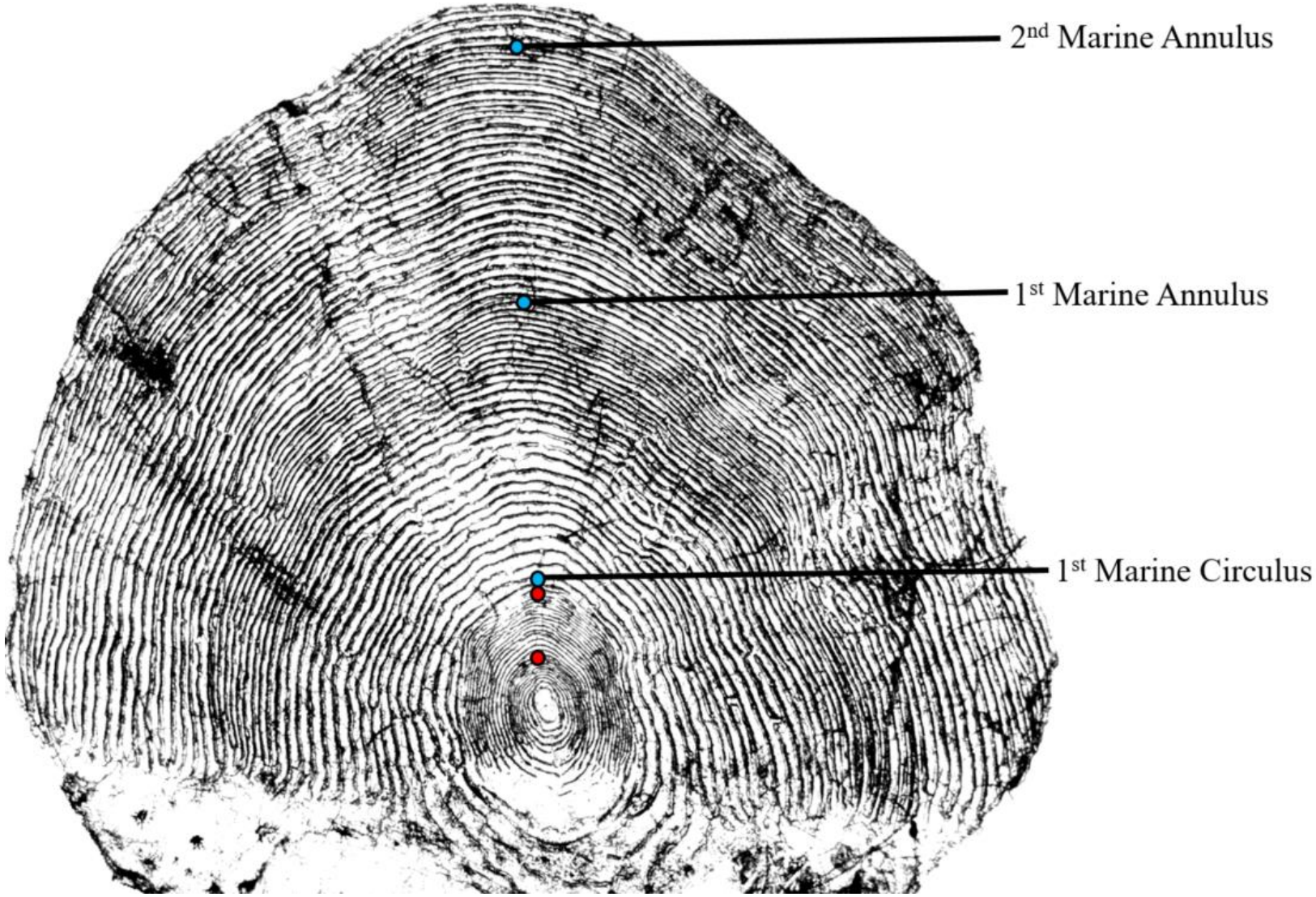
Adult returns (1SW and 2SW)



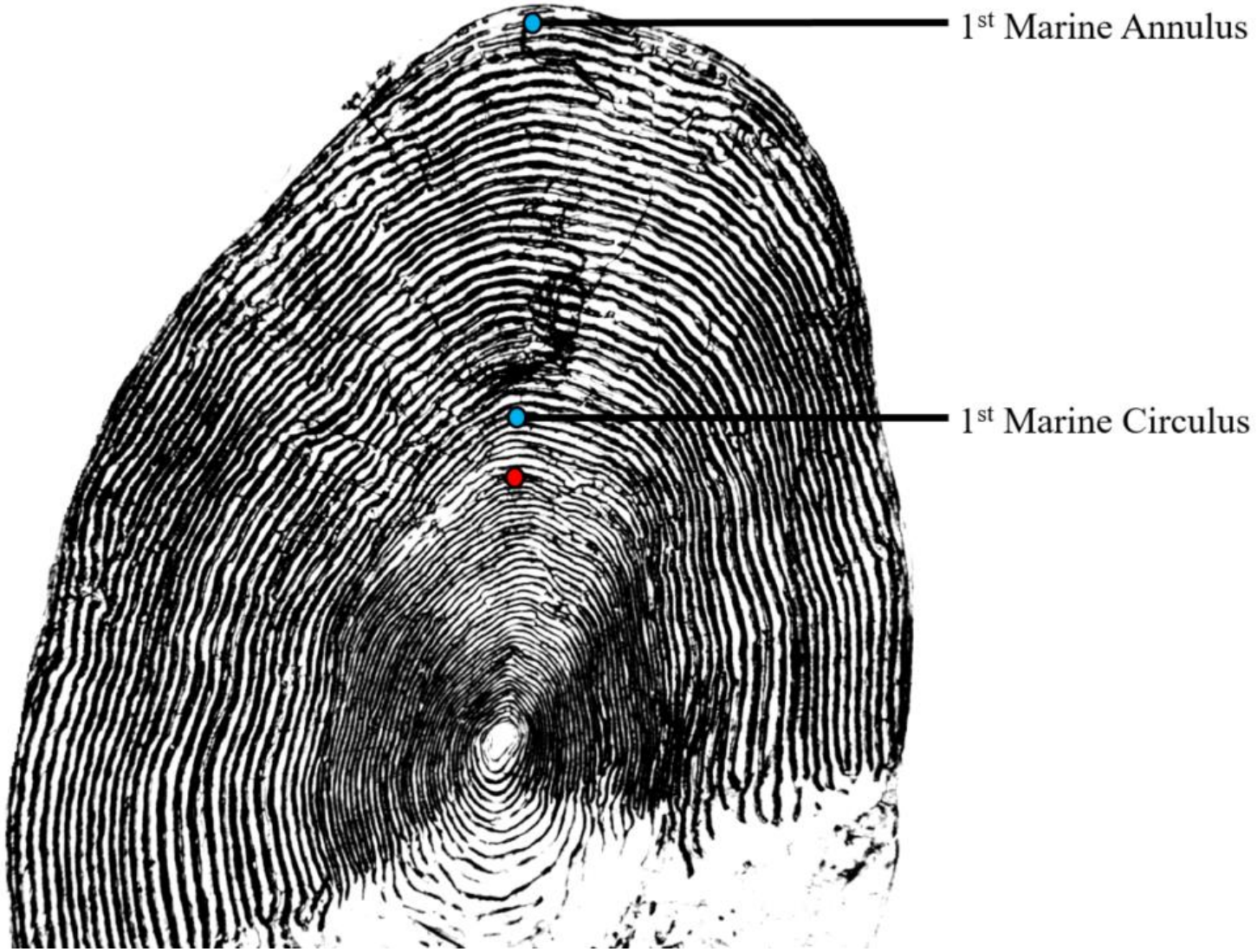
Scale from two sea-winter adult salmon sampled 18 June 2015 on the Penobscot river while returning to spawn. Freshwater annuli are marked in red. Origin determined as hatchery based on freshwater zone. Aged as 1.2.



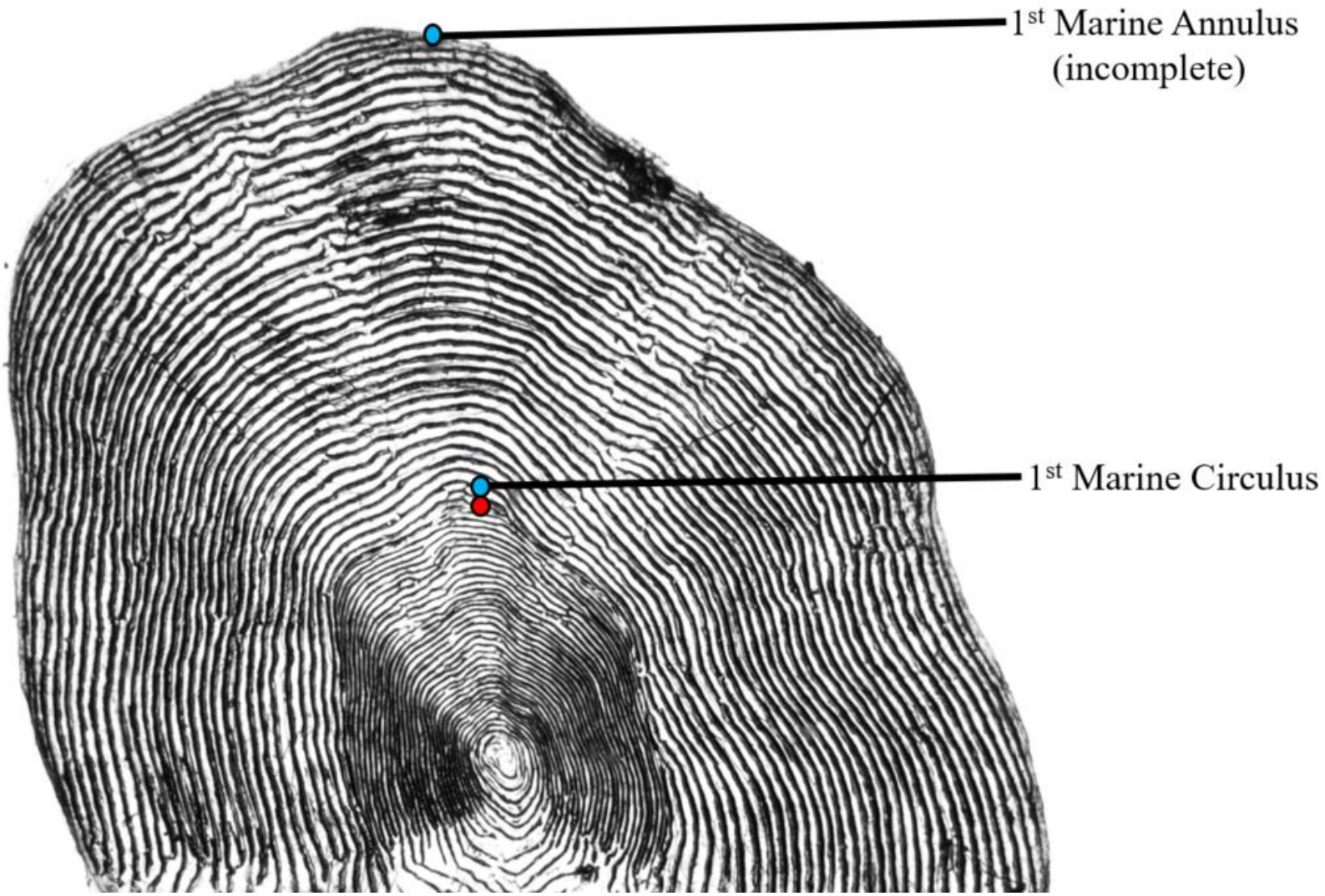
Scale from two sea-winter adult salmon sampled 25 May 2002 on the Penobscot river while returning to spawn. Freshwater annuli are marked in red. Origin determined as hatchery based on freshwater zone. Aged as 1.2. Runout present on this scale.



Scale from two sea-winter adult salmon sampled 17 June 1989 on the Penobscot river while returning to spawn. Freshwater annuli are marked in red. Origin determined as naturally reared or wild based on freshwater zone. Aged as 2.2.



Scale from one sea-winter adult salmon sampled 9 June 2015 on the Penobscot river while returning to spawn. Freshwater annuli are marked in red. Origin determined as hatchery based on freshwater zone. Aged as 1.1.



Scale from one sea-winter adult salmon sampled 17 June 2015 on the Penobscot river while returning to spawn. Freshwater annuli are marked in red. Origin determined as hatchery based on freshwater zone. Aged as 1.1. Annulus incomplete, i.e., not fully formed.

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