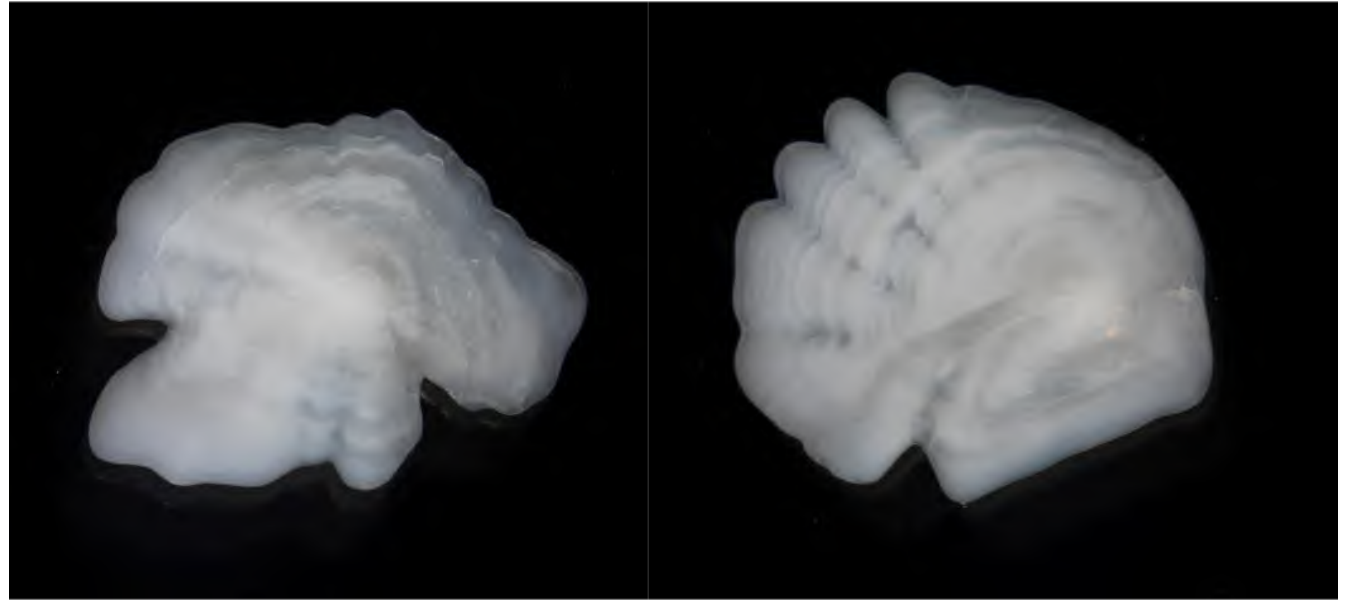


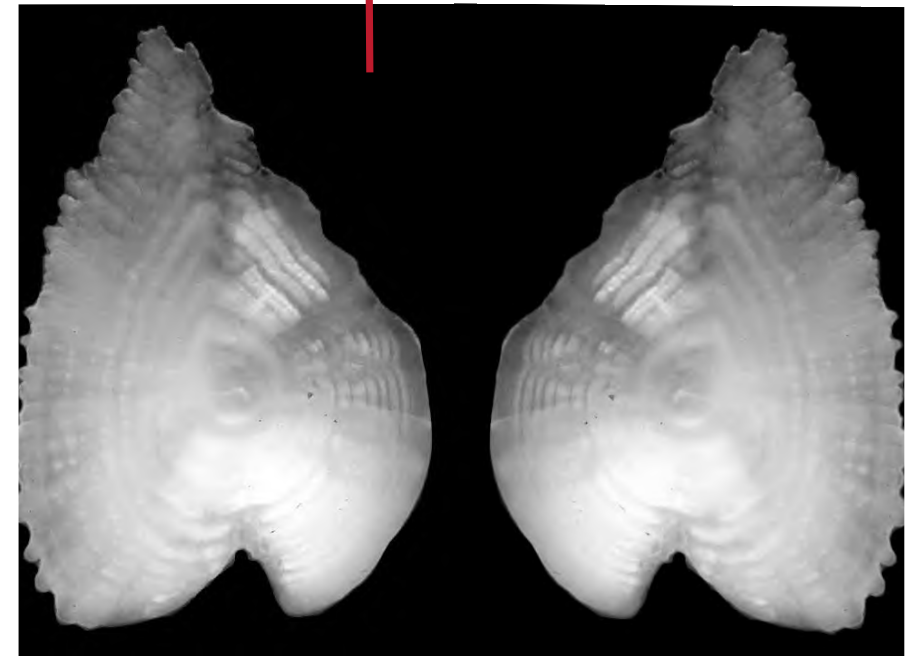
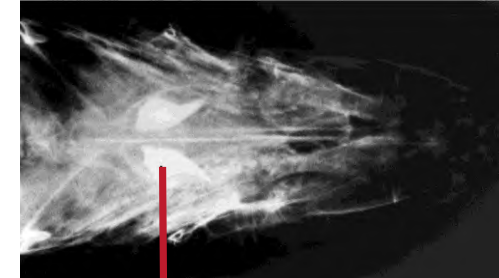
Otolith microchemistry, difficult-to-age marine species, element marking in otoliths



Tracey Loewen, Rick Wastle
Freshwater Institute
Ontario and Prairies Region

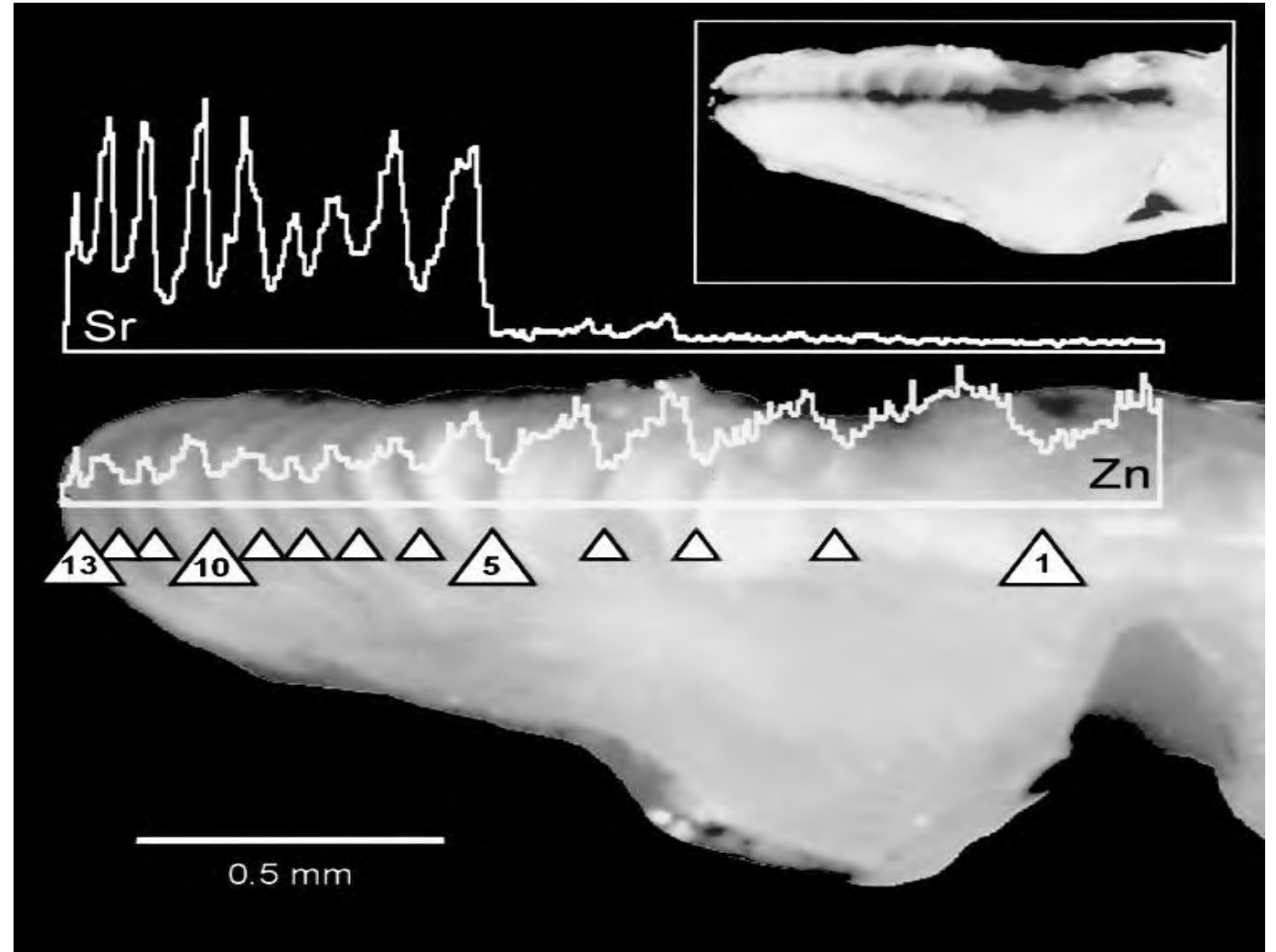
FWI Otolith Age Estimation Lab

- 3 FTE's and 1 seasonal FTE on our team.
- Primary work of the group is to provide age estimates to DFO clients within our region.
- Provide age estimations for approximately 32 different species (freshwater to marine with primary focus on Arctic Char, Dolly Varden Char, Lake Trout, Greenland Halibut, Redfish (new as of 2023), Arctic Cod).
- Provide training and education to students upon request.
- Support research and development when possible.
- Development of otolith physical archive, policies on access to the physical archive and associated electronic database (good data management practices)



Otolith Microchemistry to support Age estimation and validation: Case study Arctic Char

- Element variation (Sr anadromous species who migrate between fw and sw)
- Zn (Halden et al. 2000) –salmonid species
- Other element and isotope uses (oxygen, carbon, strontium)

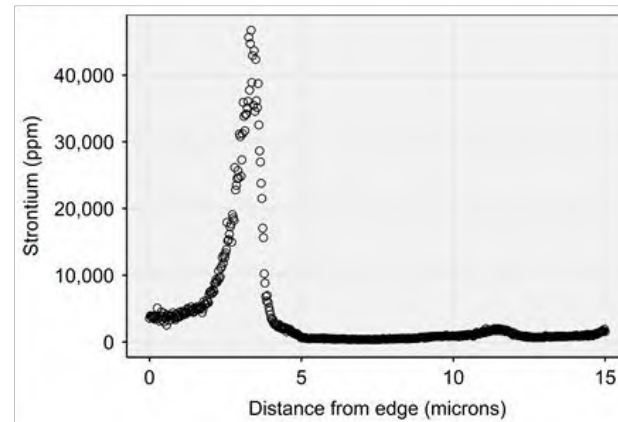
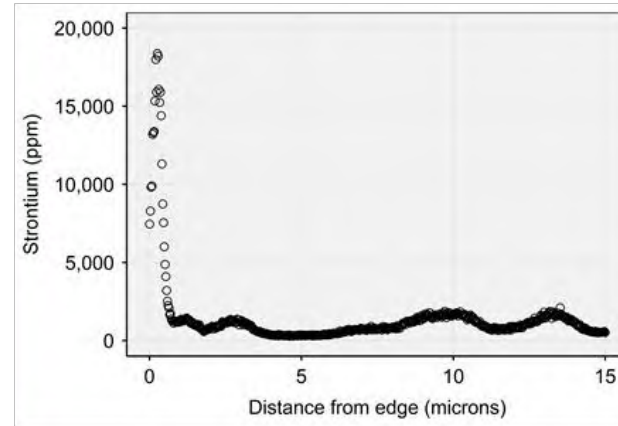
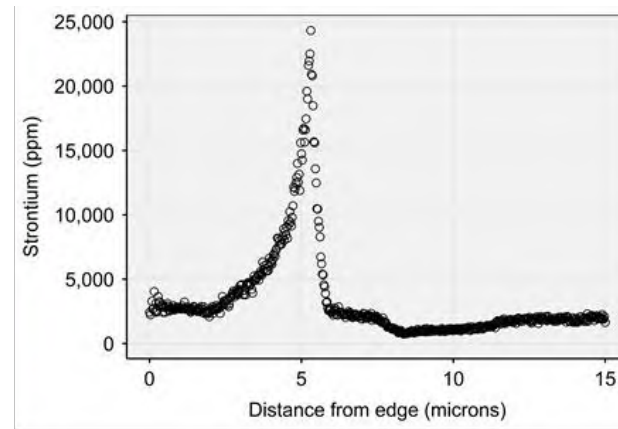


Halden et al. 2000

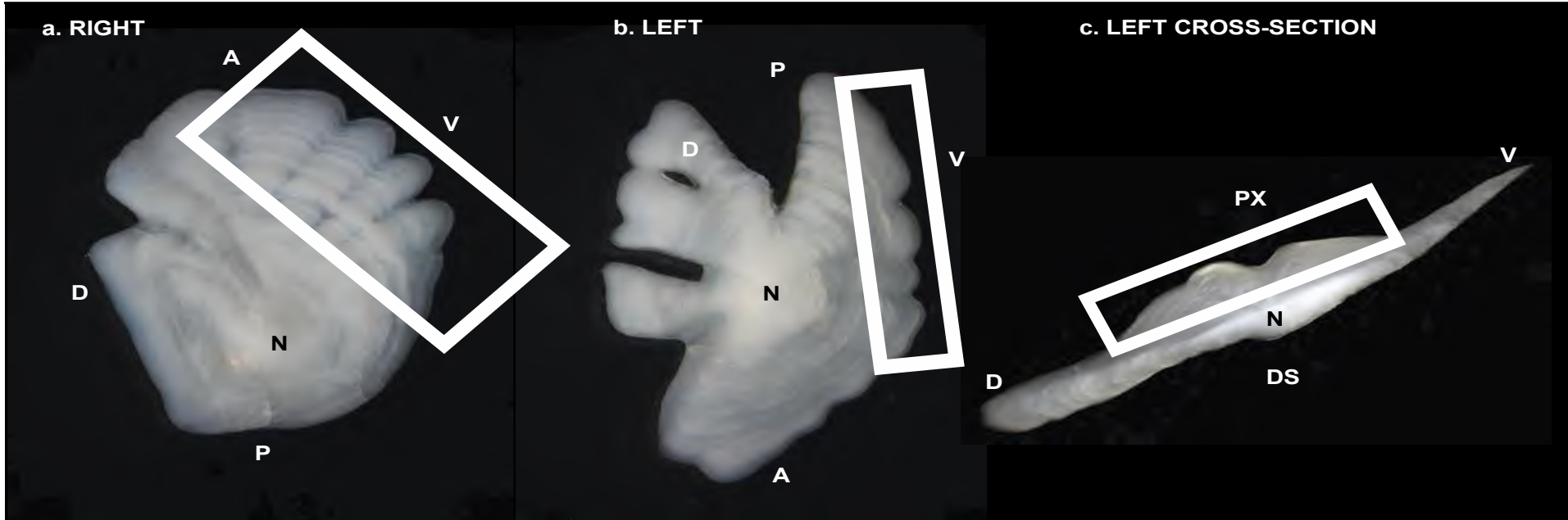
Otolith Element Marking –Age validation: Case Study

Arctic Char, Cambridge Bay

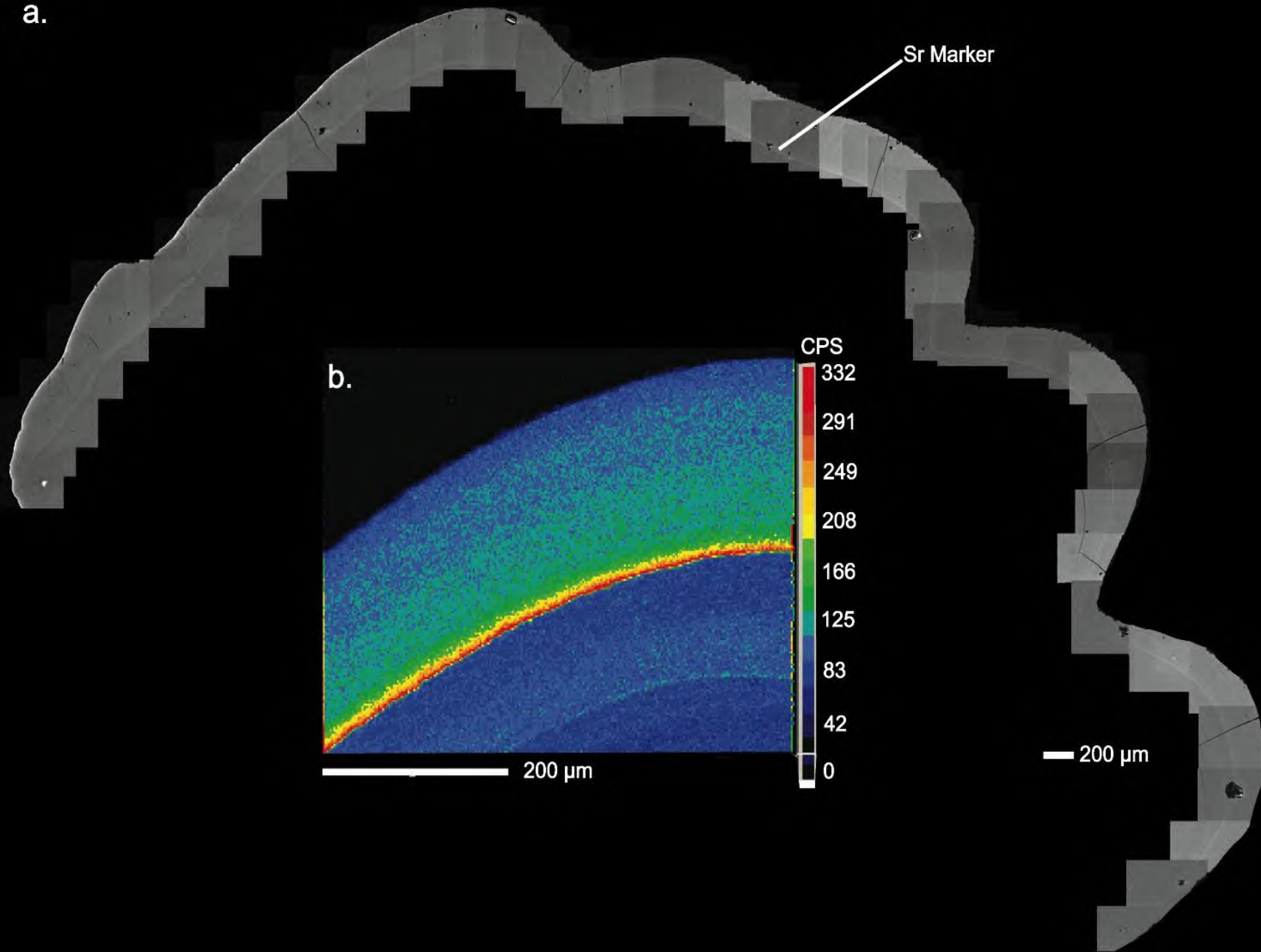
- SrCl spiking in wild fish
- Returns in the fishery
- Arctic Char (C. Gallagher) and Greenland Halibut (M. Treble)
- Comparisons of dosing examined – low dosed individuals may show a signal using EDS but not with the BSE imaging – contrast is not significant to show up in the imaging
- SEM–BSE mode using EDS (Energy Dispersive Spectroscopy) to examine semi-quantitative element intensity plots (counts per second)
- Things to consider – otolith preparation for imaging – carbon coating (machine parameters influence optical viewing of signal)



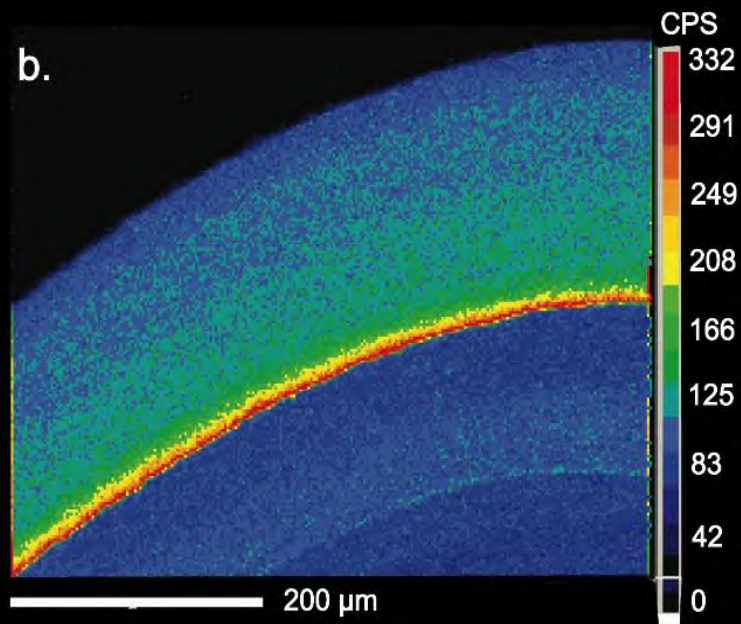
Primary Researcher: C. Gallagher
 Collaborations: A. Lussier, CMN



a.

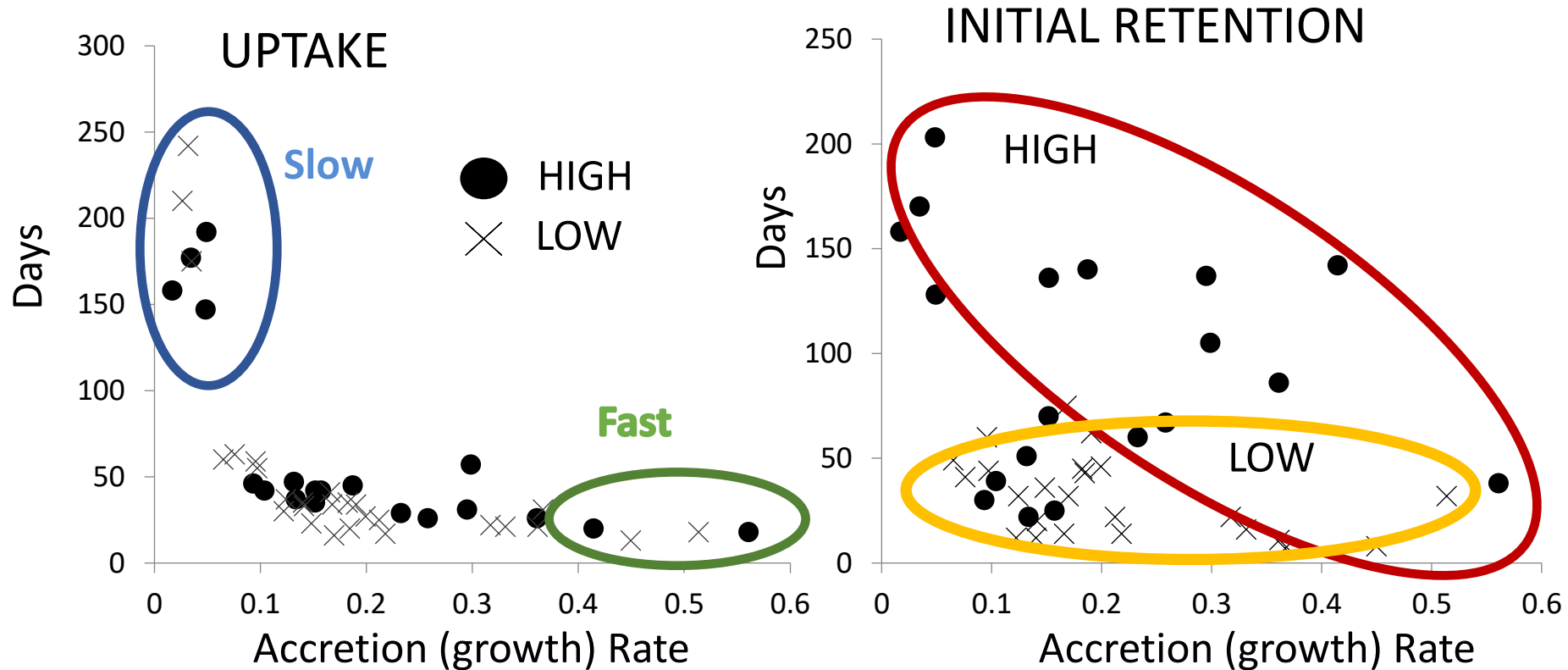


b.



200 μm

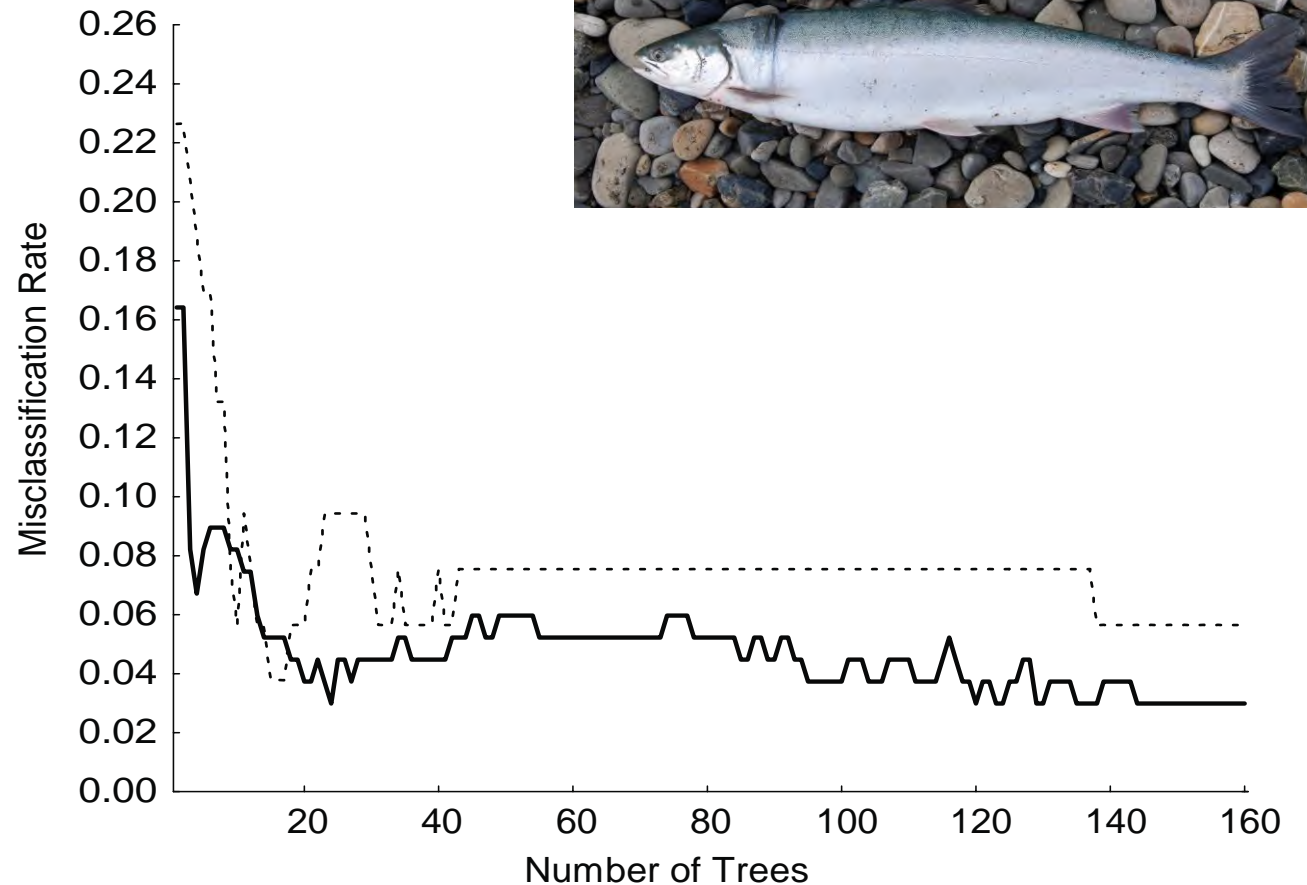
Element Marking

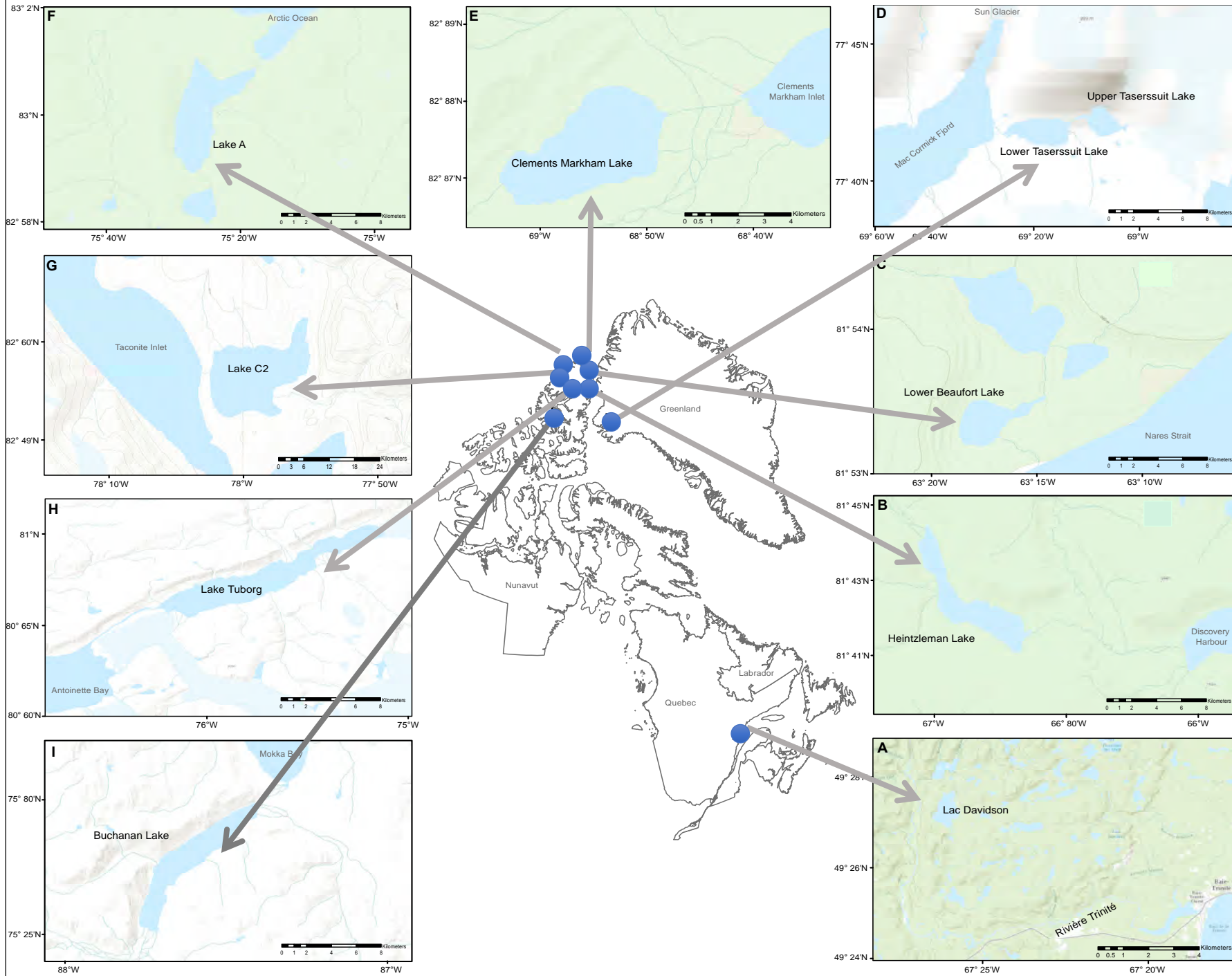


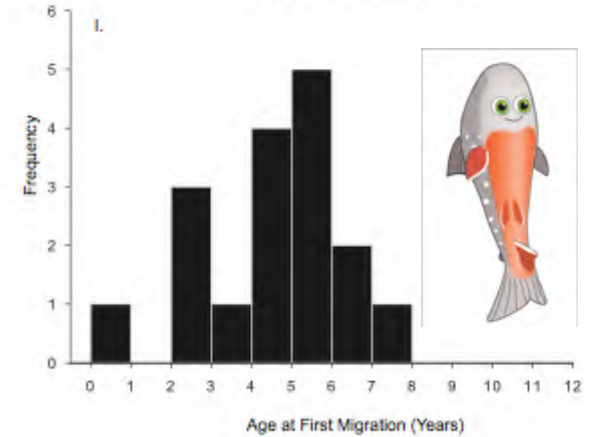
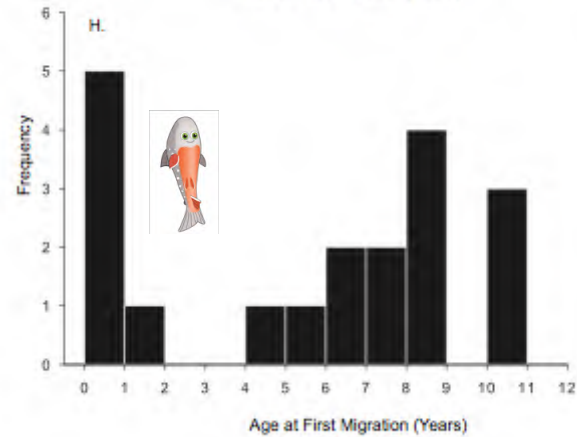
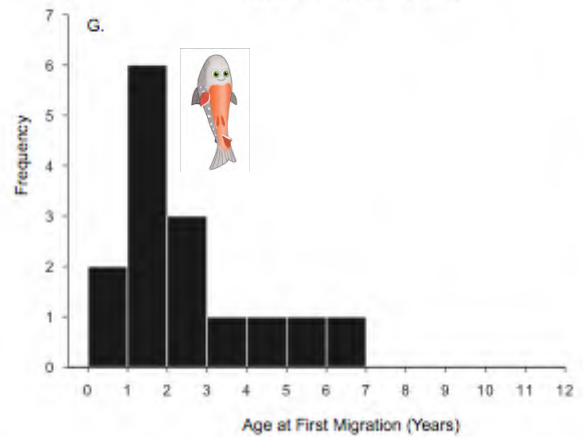
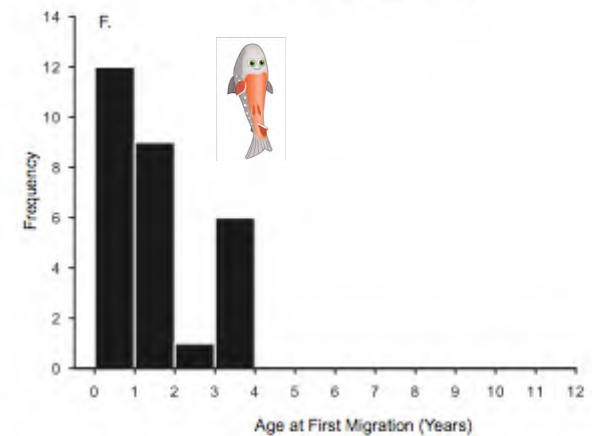
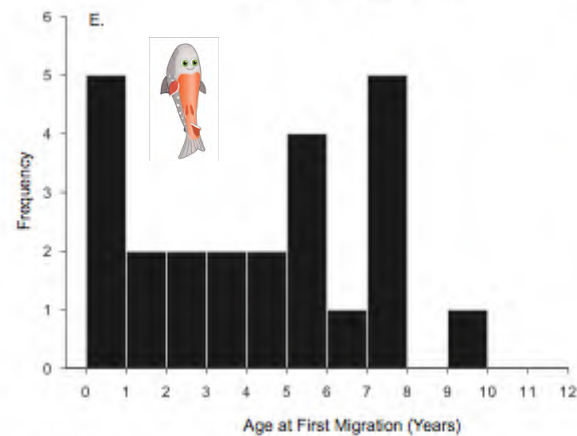
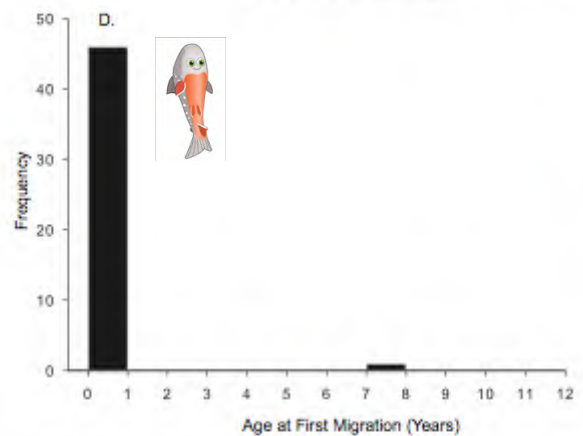
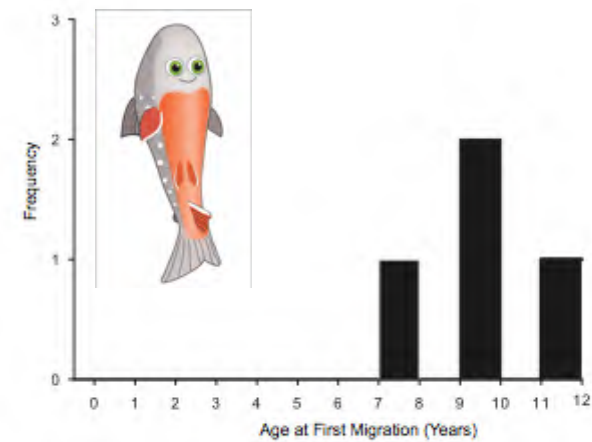
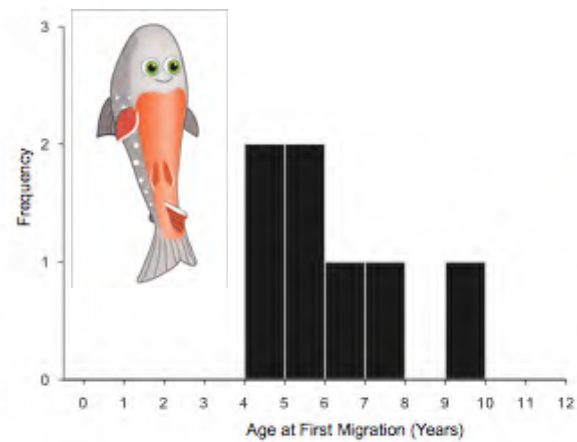
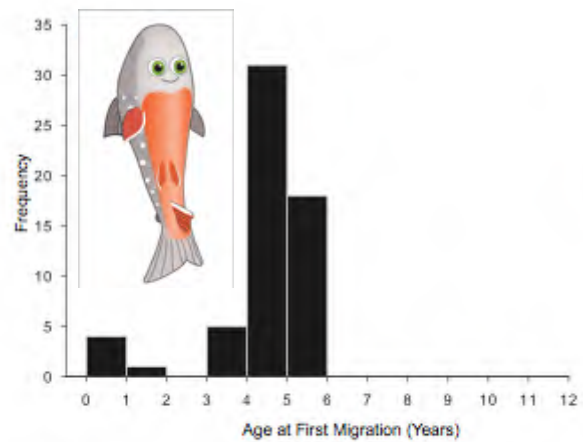
- Uptake 17-242 days
- Otolith accretion (growth) rate and dose play a role in the initial stages of uptake
- Dose impacts the initial retention period where the higher dose took longer to reach a point of stability

Otoliths and other uses of microchemistry for stock assessment

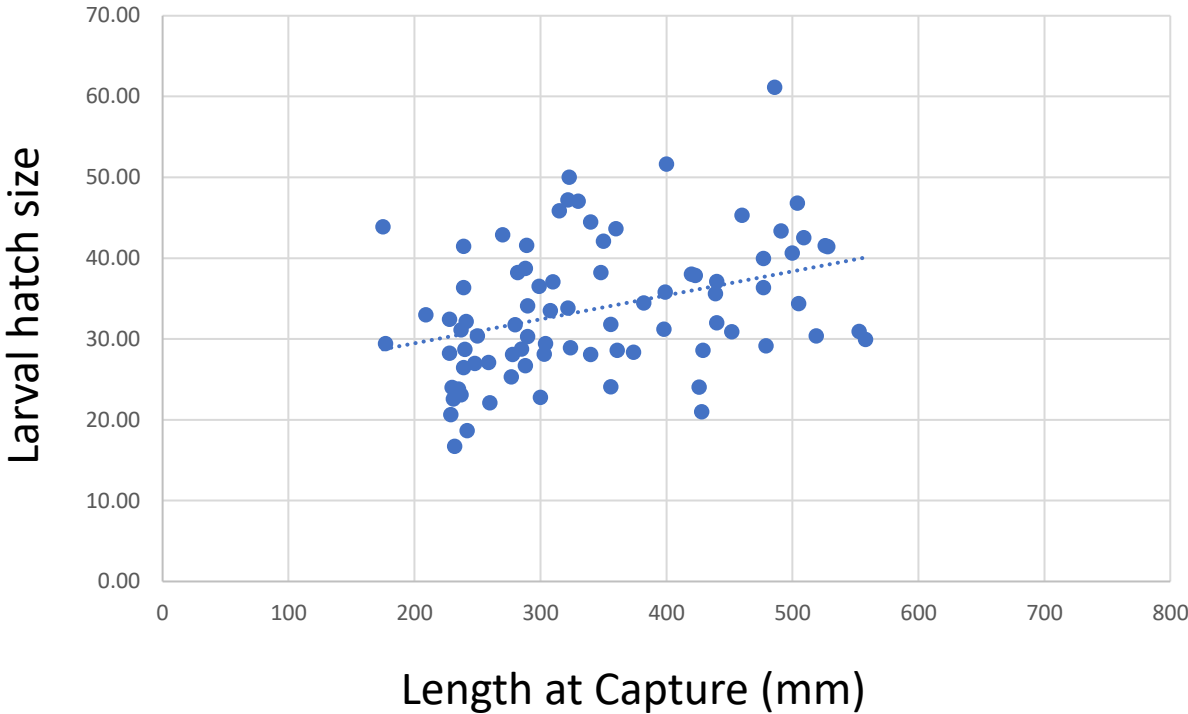
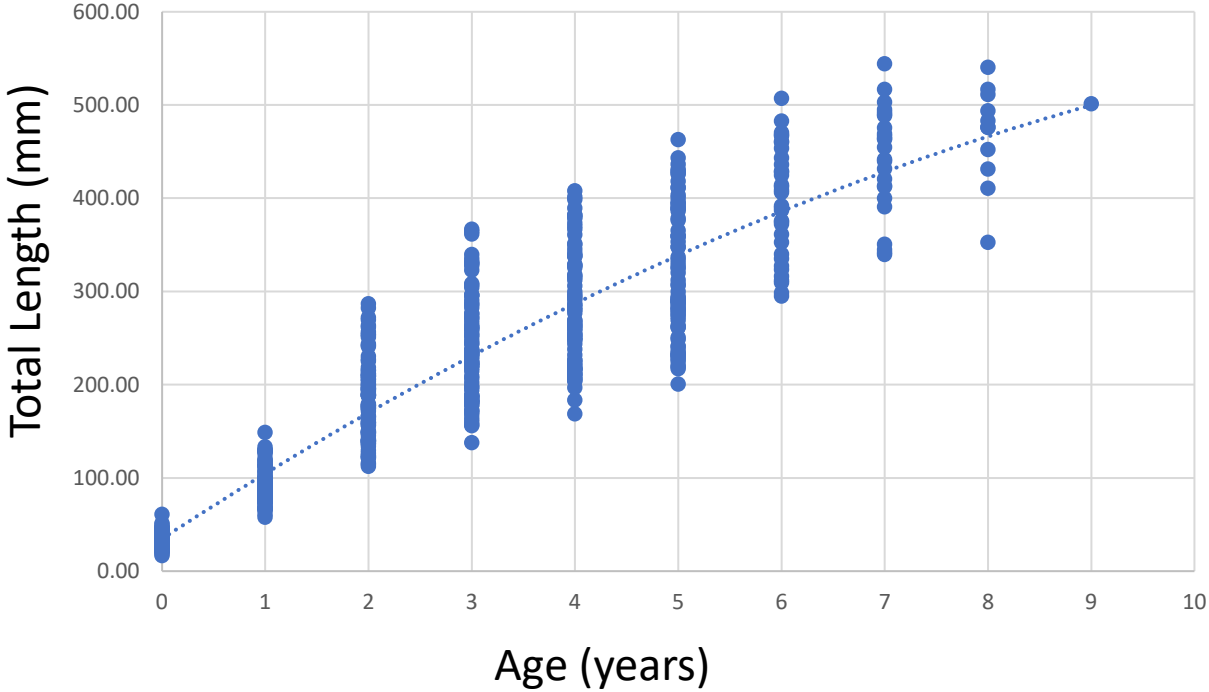
- Stock discrimination (Sr isotopes)
- Life history bio-demographics







Uses of Otolith increment marking to better understand age structure: Case Study Greenland Cod





Fisheries and Oceans
Canada

Pêches et Océans
Canada

A New Method for Estimating Ages of Greenland Halibut – the Left Otolith Bake and Longitudinal Thin Section Method

by

Rick Wastle

TESA workshop "Best practices in ageing": Moncton
New Brunswick, Jan. 31 – Feb. 2, 2023



Canada

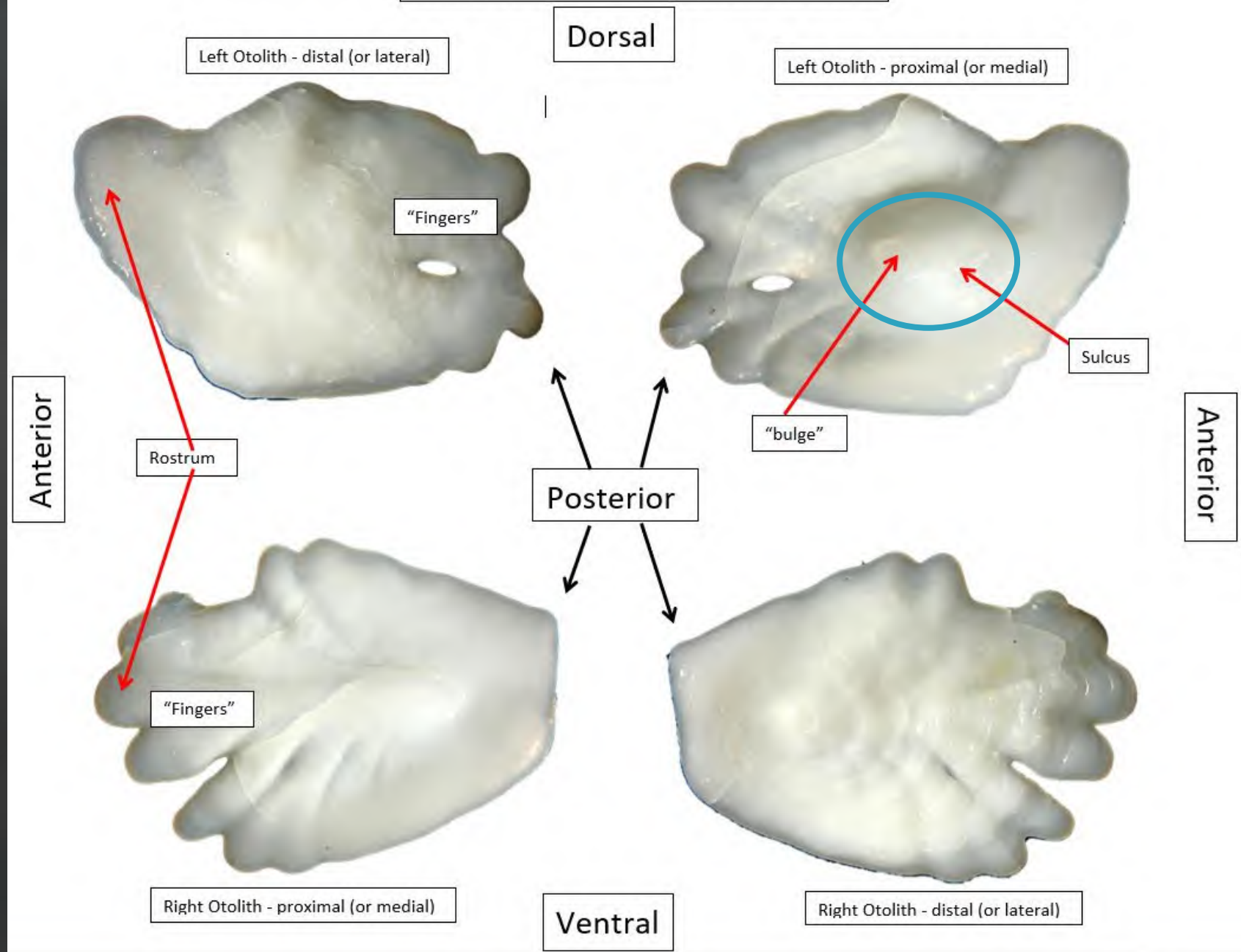
Housekeeping

- In order to discuss a new approach to Greenland halibut otolith age reading, I need to first talk a bit about some of the methods used previously
- I'll be using the term "bulge" a lot in this presentation when referring to the protuberance found in the middle of the proximal side of the left otolith
- "centro-proximal protuberance"
- We read these otolith thin sections using reflected light, with the sample in water with a black background

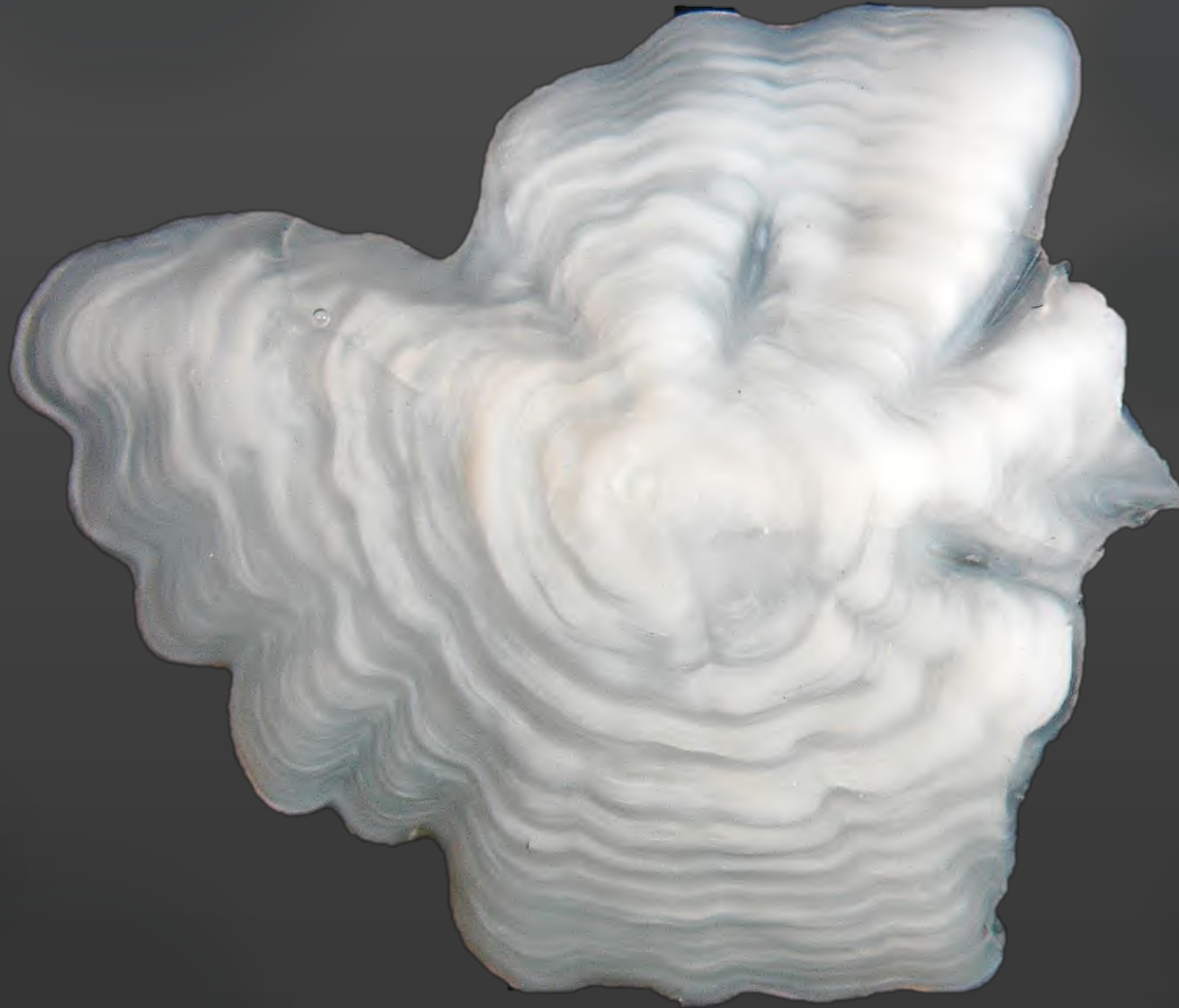
Methods Attempted at DFO Winnipeg

- Whole otolith (L and R) – reflected light, in water
- Whole otolith (L and R) – HCl treated, reflected light, in water
- Whole otolith (L and R) – transmitted light, in water
- Whole otolith (L and R) – baked, reflected light, in water
- Transverse sectioned L otolith – no treatment, stained, and baked before sectioning, (reflected and transmitted light)
- Grind & burn L otolith
- Vertebrae – no treatment, stained and baked
- Scales – polarized transmitted light
- Various fins – thin-sectioned, reflected light
- Opercula bones – reflected light

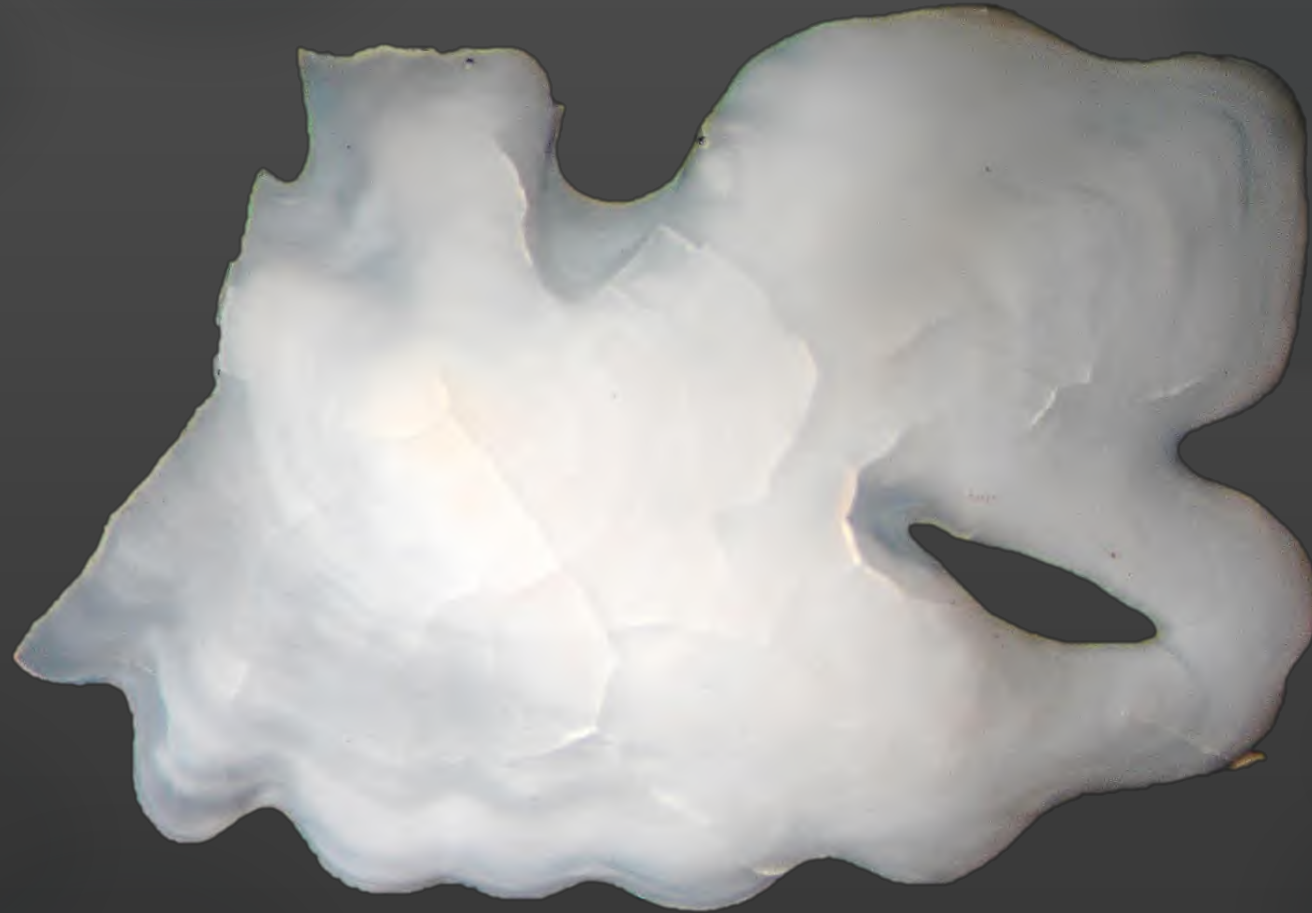
GREENLAND HALIBUT OTOLITHS



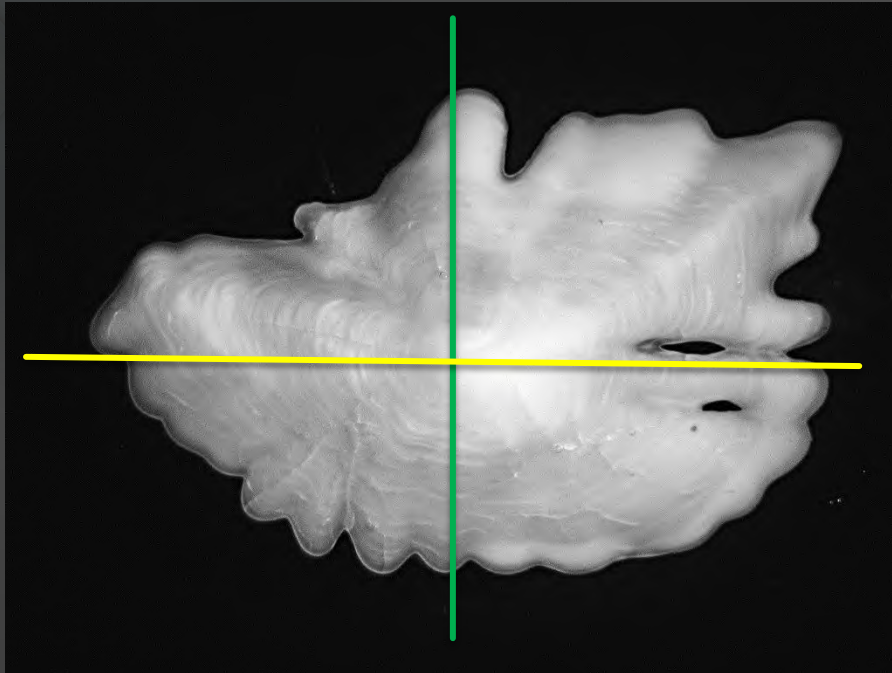
46 cm female; age estimate 12+



46 cm male; age estimate ??



Greenland Halibut Left Otolith Section Planes

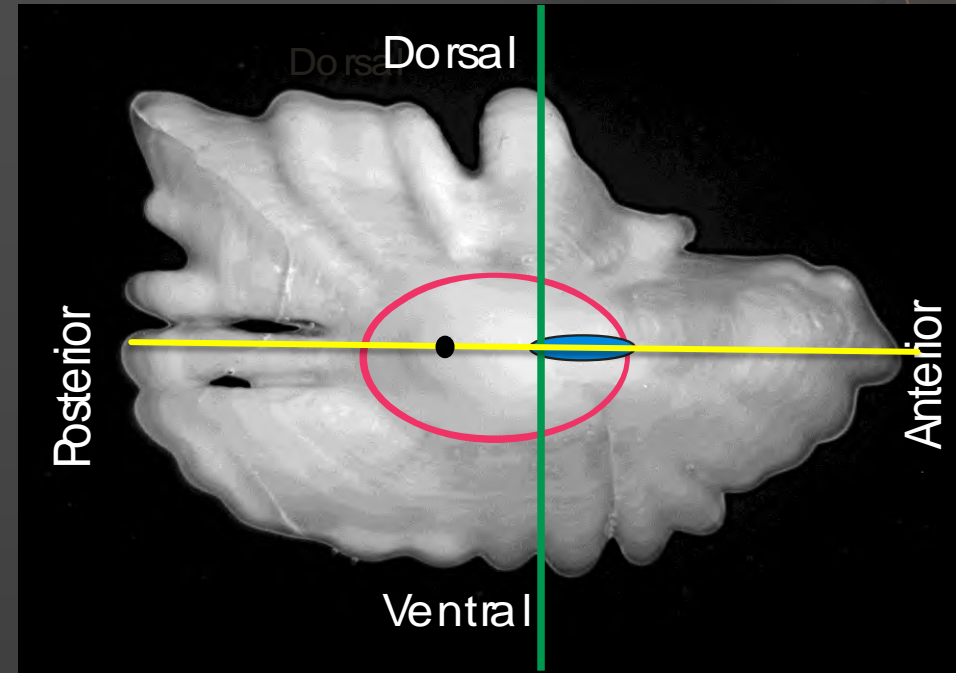


Distal View

Section Planes through the nucleus

Green line – transverse

Yellow line – longitudinal



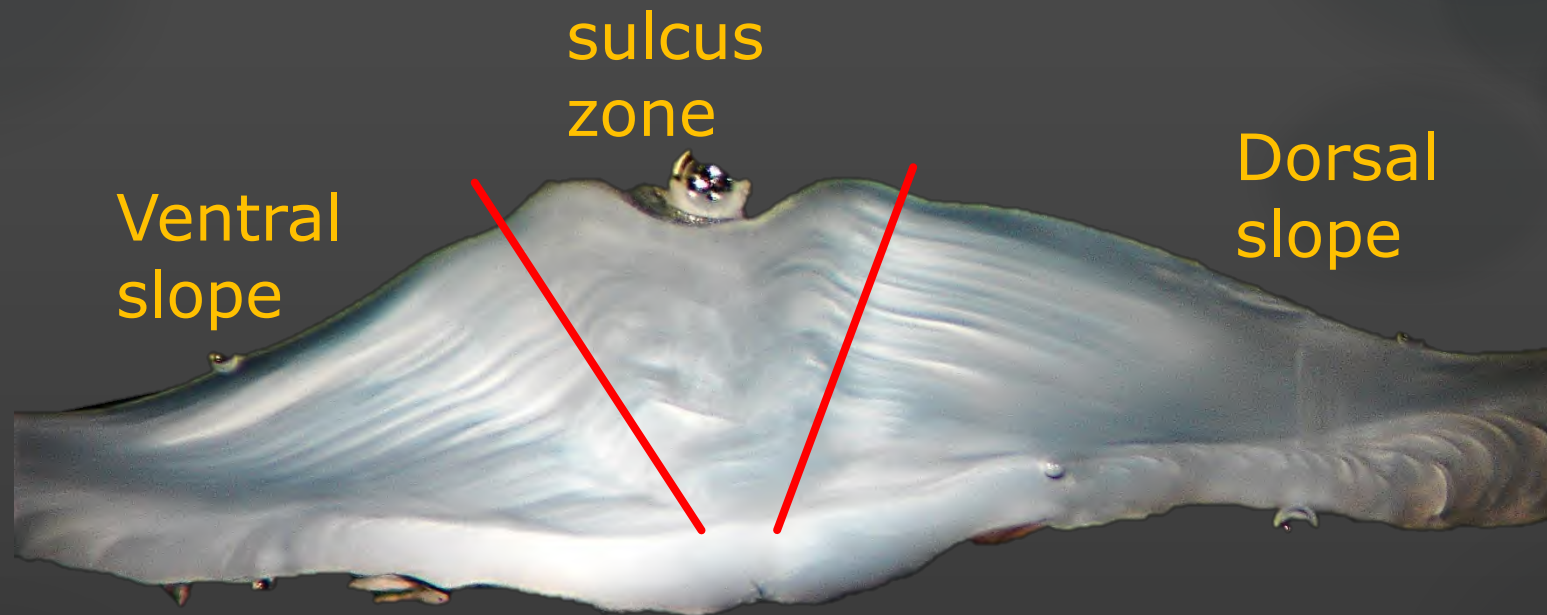
Proximal View

Red Oval – approx. location of the bulge

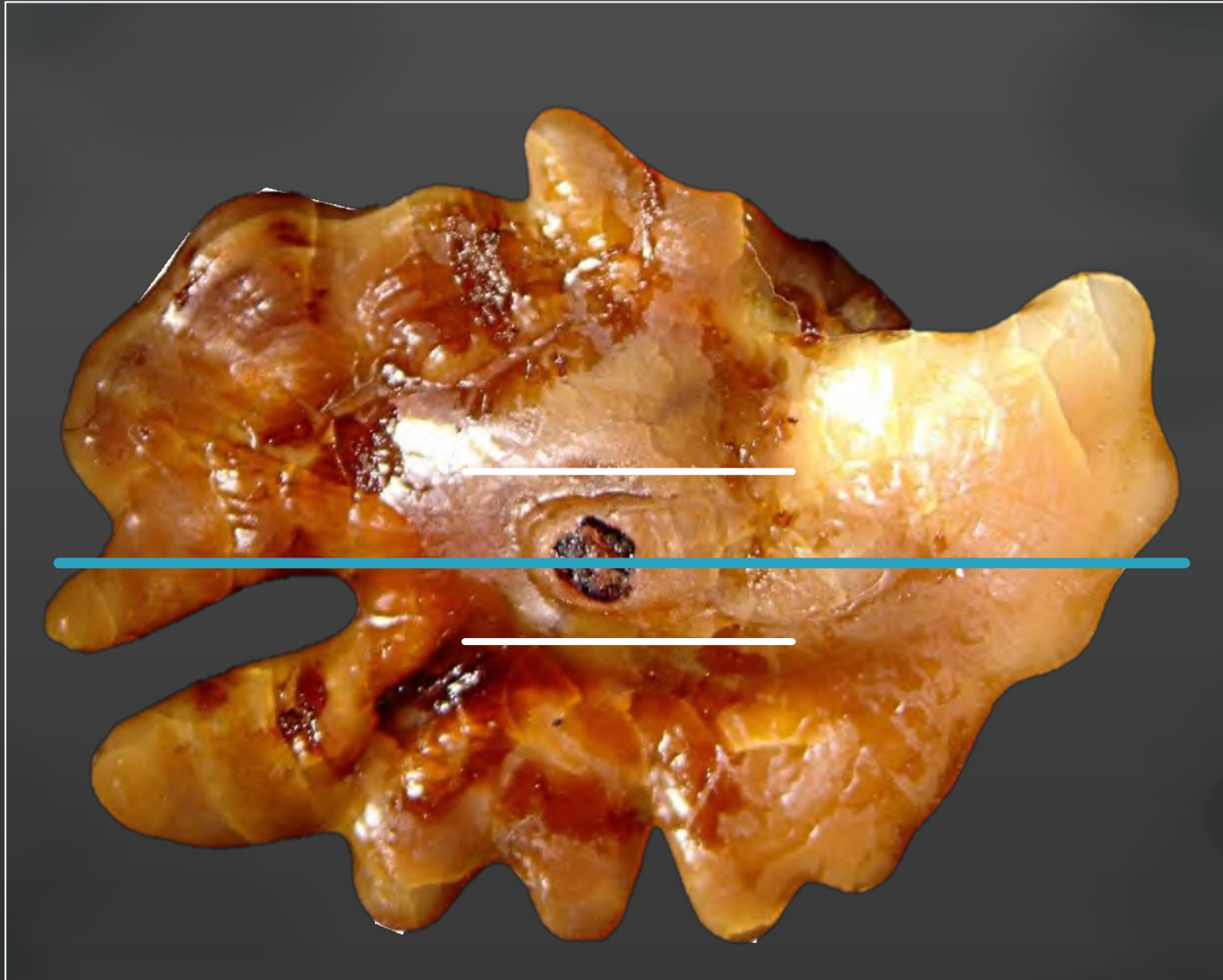
Blue Solid Oval – approx. location of the sulcus

Black Dot – “peak” of the bulge

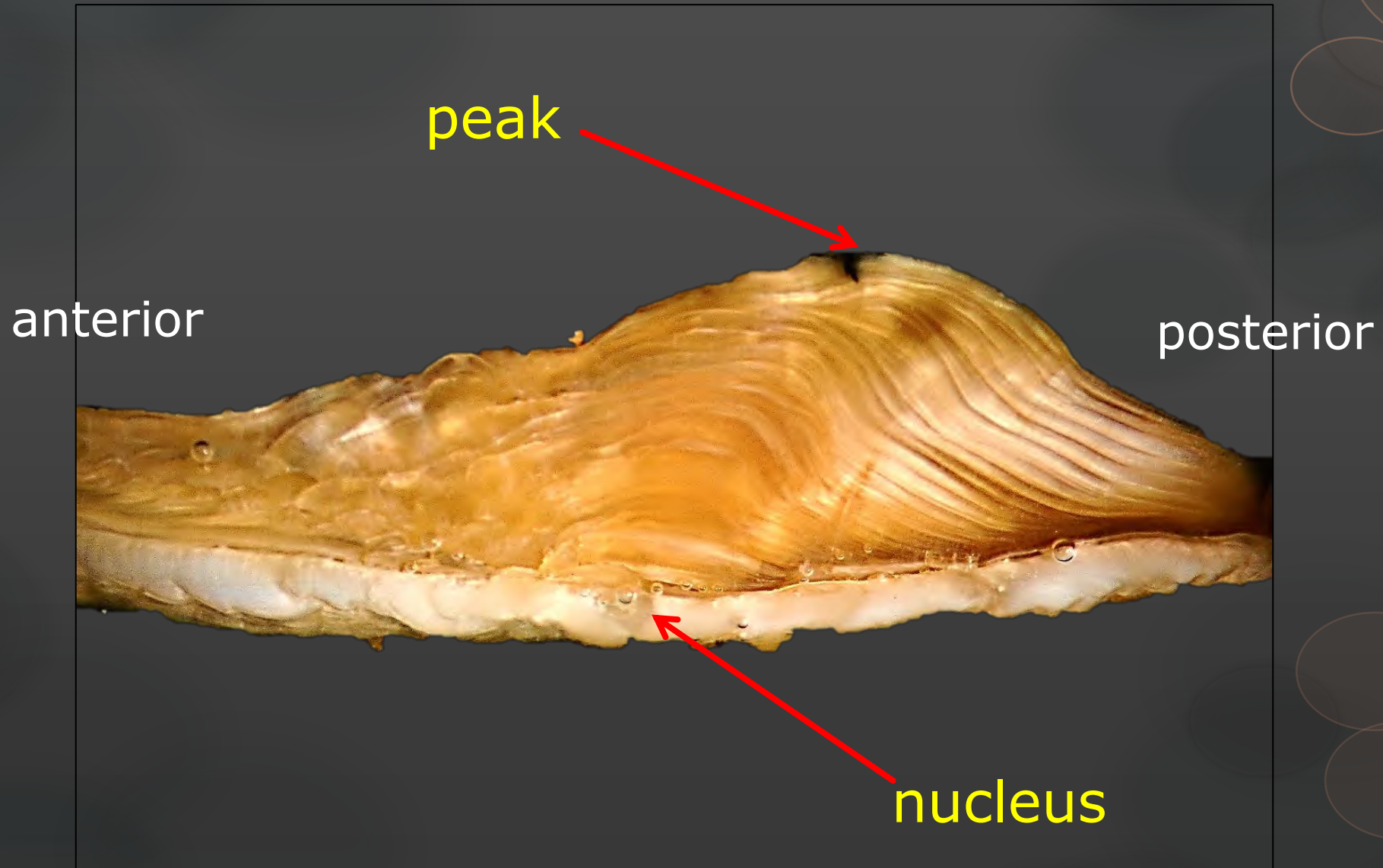
The Left Otolith Bulge Transverse Cross-section can be Divided into 3 Zones



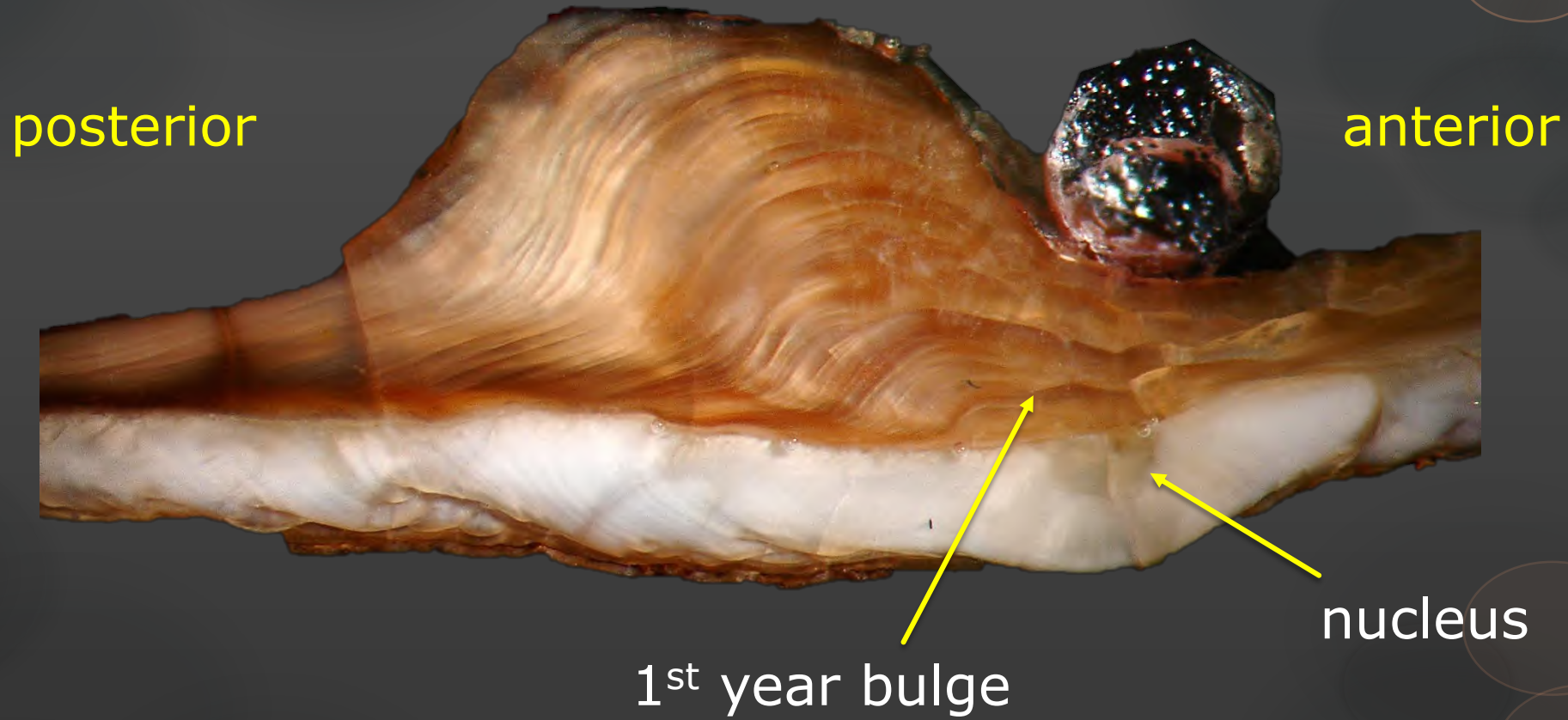
Baked Left Otolith (proximal view) - Using the Ridge at the Top of the Bulge to Choose where to Section



Baked Left Otolith Bulge Longitudinal Section



1st Year Profile - Hitting The 1st Year Bulge



1st Year Profile – 1st Year Bulge Missed

posterior

anterior



no nucleus

1st year

Lear & Pitt (1975)

290

J. FISH. RES. BOARD CAN., VOL. 32(2), 1975

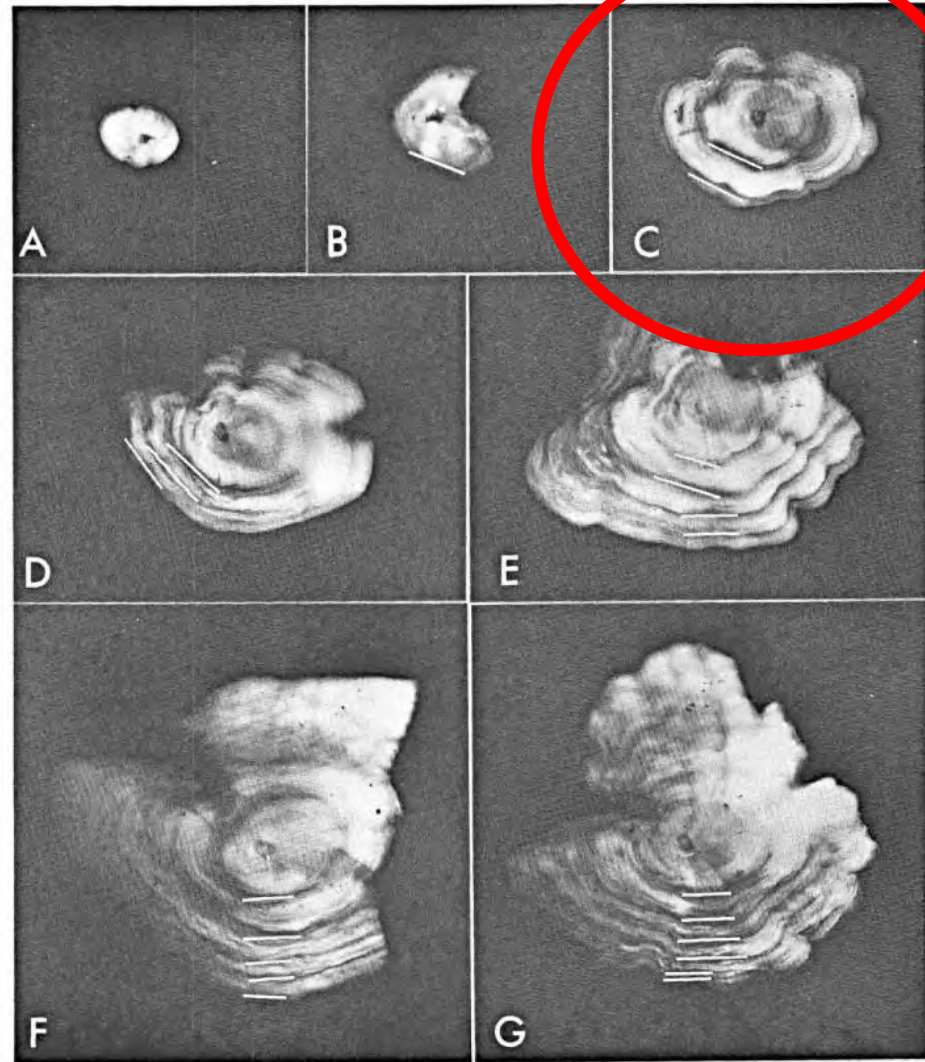
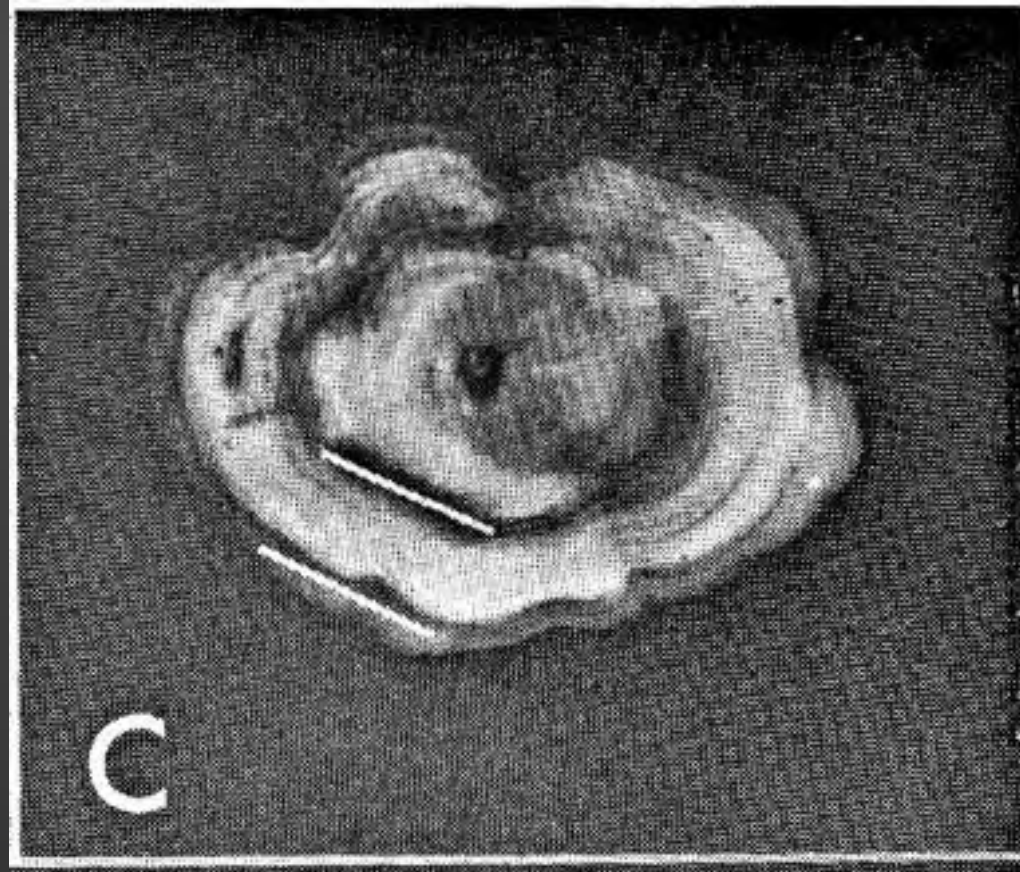


FIG. 1. Representative otoliths of the ages 0+–6 yr inclusive ($\times 8$). A, age 0+, 7.1 cm October 21, 1967; B, age 1, 12 cm April 23, 1967; C, age 2, 16 cm October 23, 1967; D, age 3, 21 cm October 23, 1967; E, age 4+, 33 cm October 30, 1967; F, age 5, 36 cm January 16, 1968; G, age 6, 41 cm January 16, 1968. The marks indicate the center of the hyaline zone of each annulus. All the otoliths except B are from the left side of the fish.

Lear & Pitt (1975) – 16 cm fish
caught Oct. 23, 1967. Age 2.



Lear & Pitt (1975)

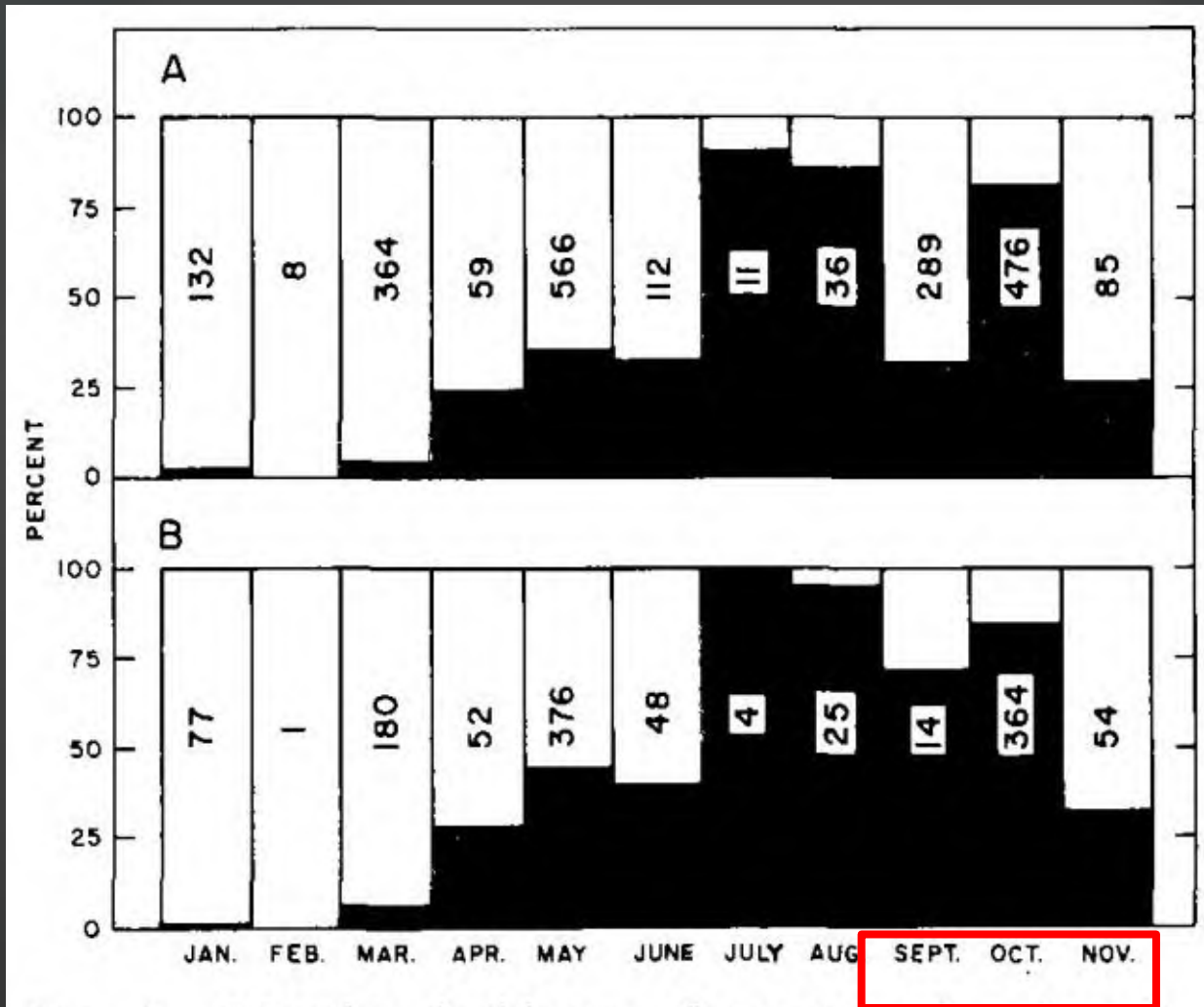
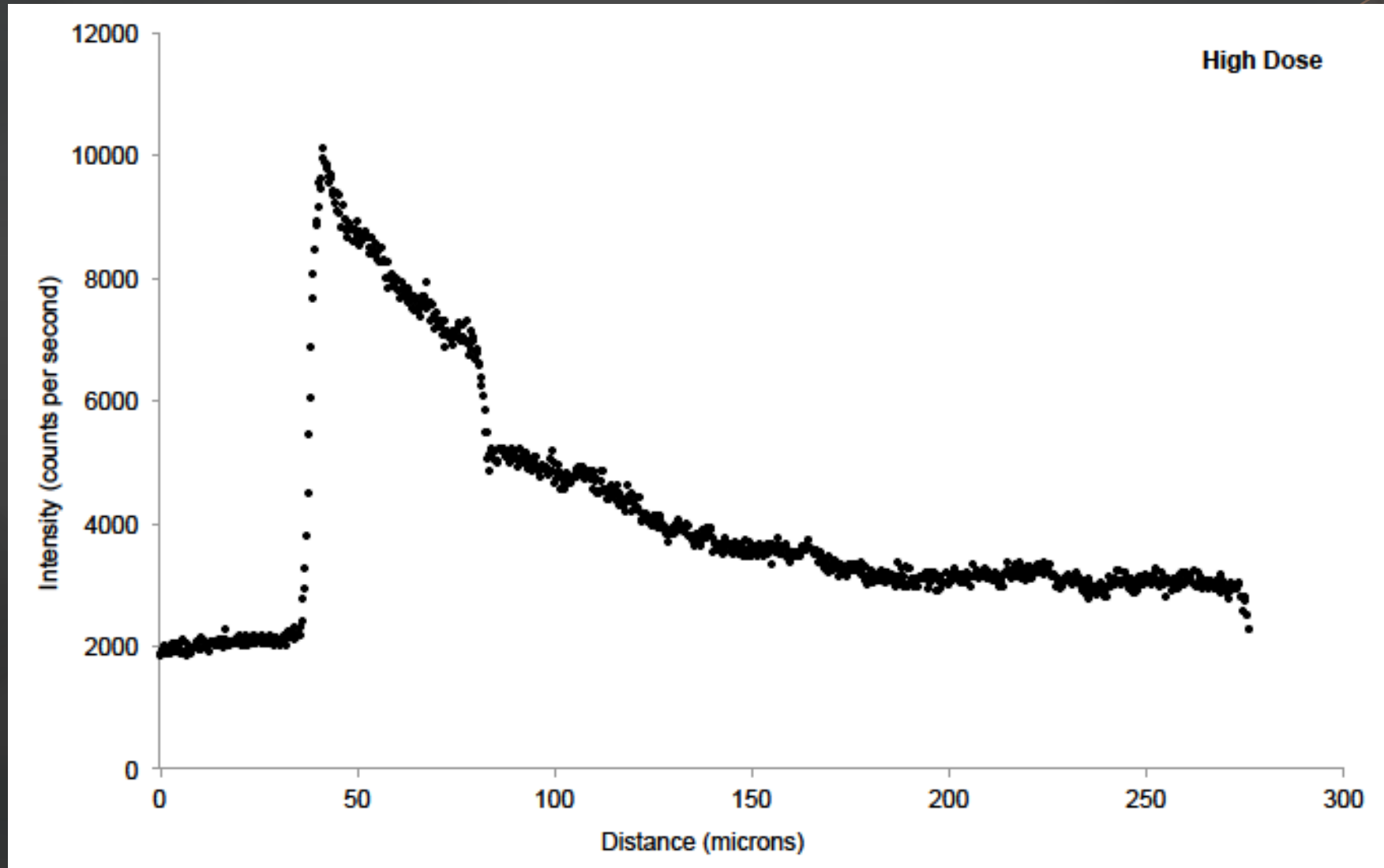


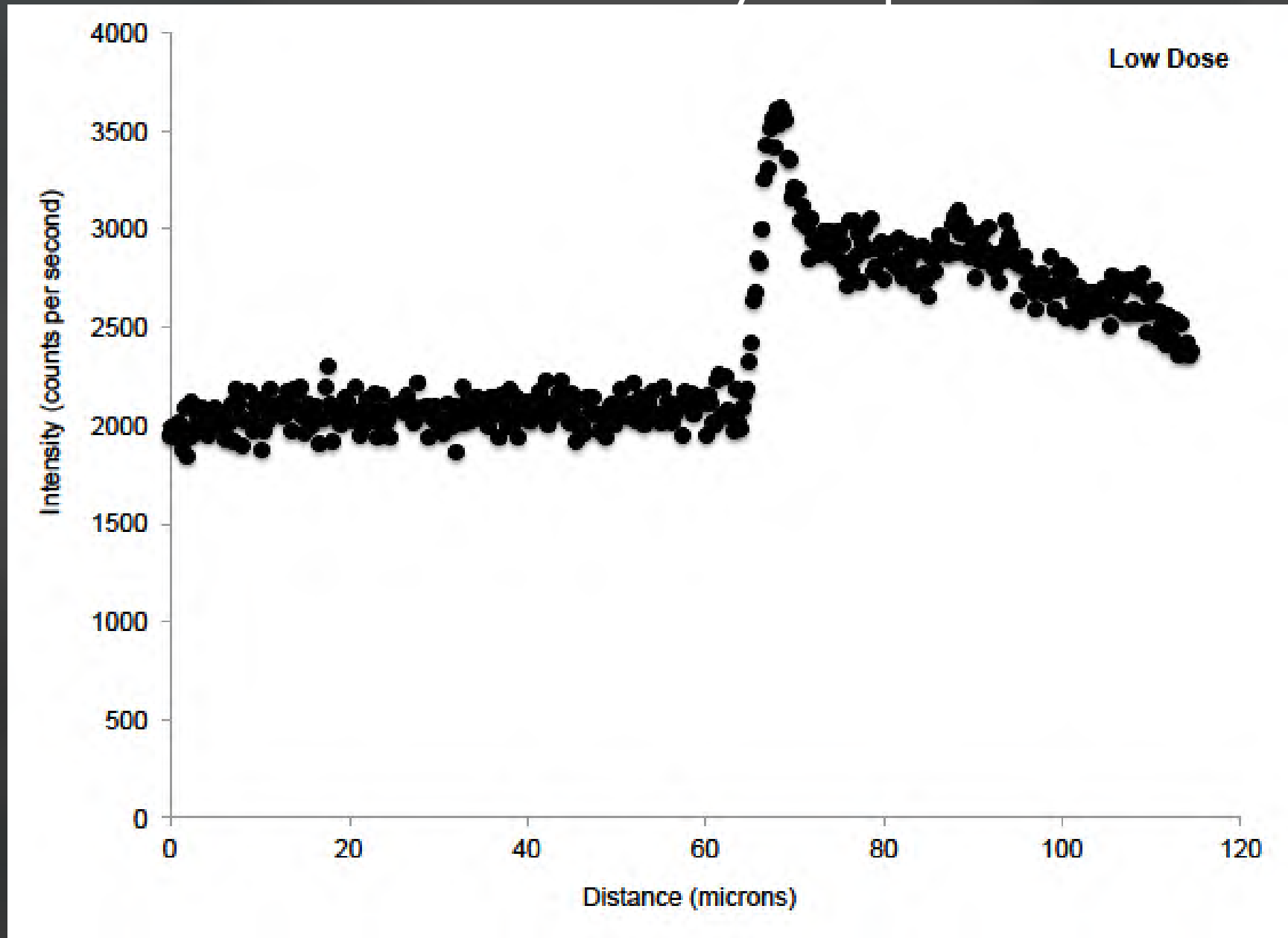
FIG. 3. Monthly incidence of opaque edge deposits on otoliths from all areas for: A, fish of all ages, and B, fish of age 0+–6 yr inclusive. Numbers of fish are shown.

Preliminary Validation Work

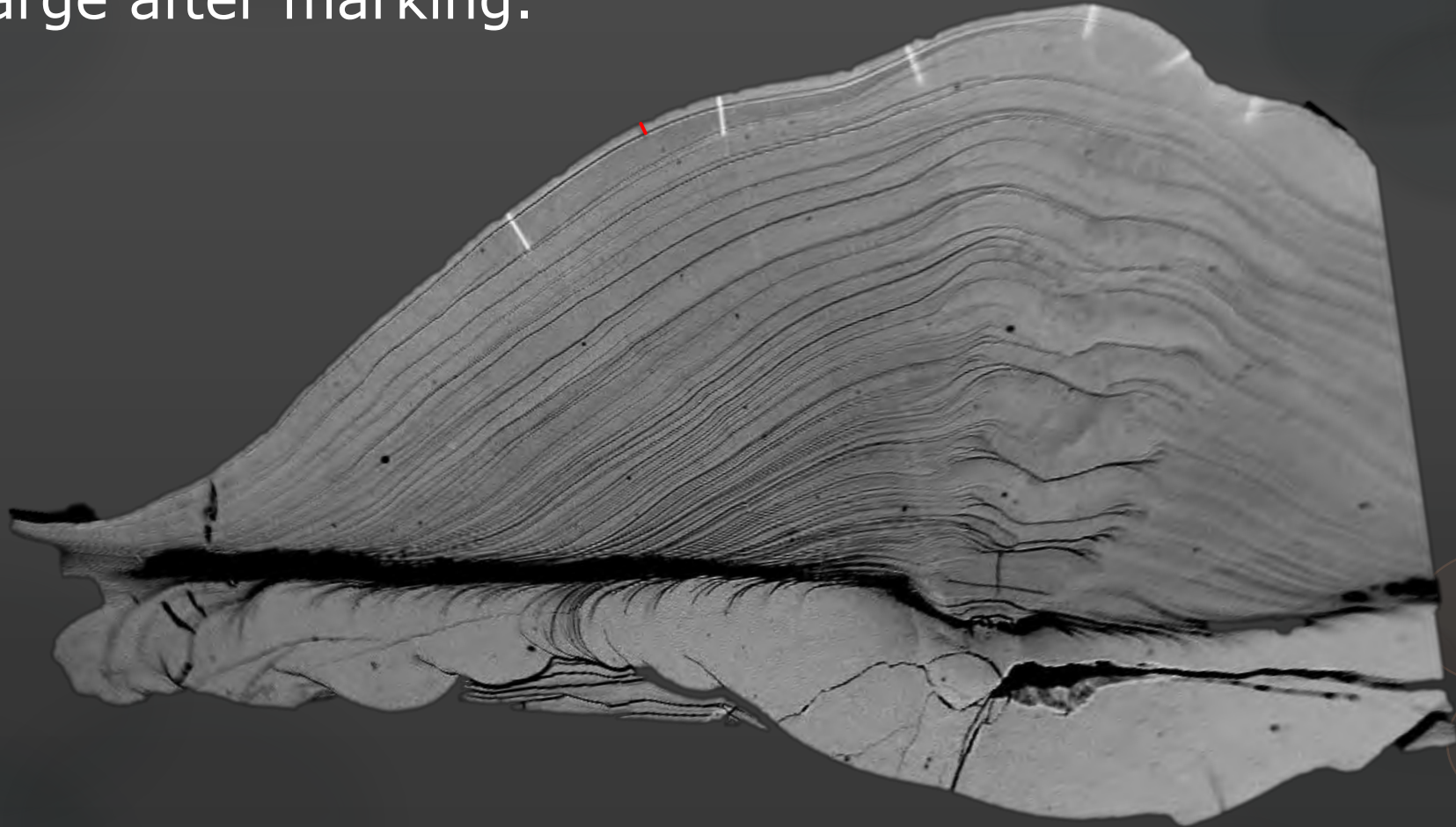
High Dose SrCl Injection (100mg/kg) – Strontium Count Intensity Graph



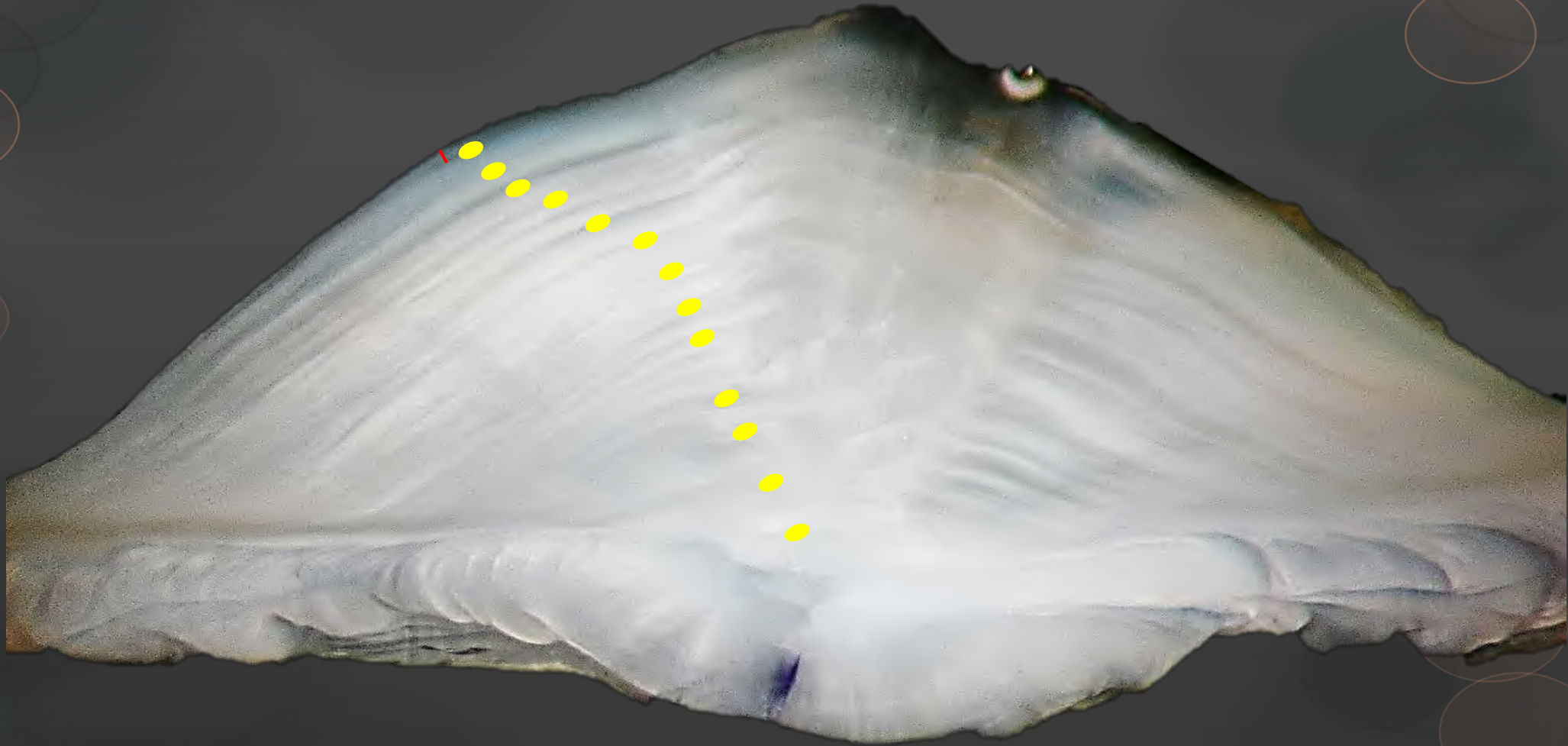
Low Dose SrCl Injection (20mg/kg) – Strontium Count Intensity Graph



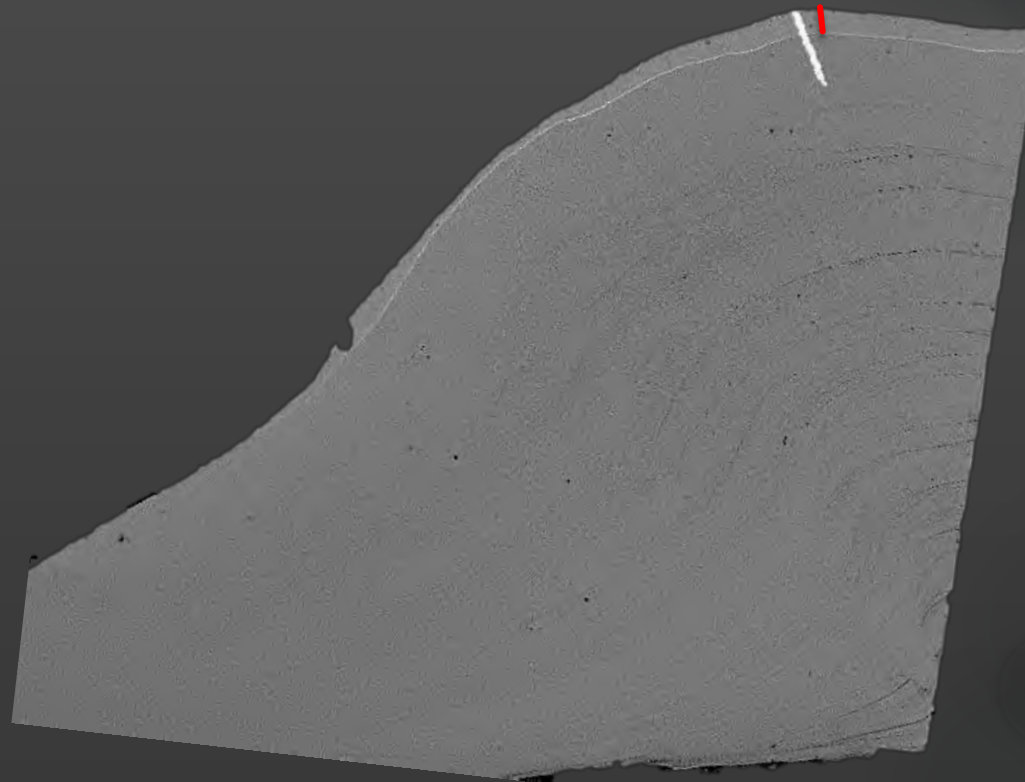
#6402 (high dose) - Left Otolith Bulge Transverse Thin Section - Back Scatter Electron Image with a bar (red) indicating growth after the SrCl mark. Tagged Oct. 9, 2009; recaptured May. 16, 2010 (53 cm). 7.3 months-at-large after marking.



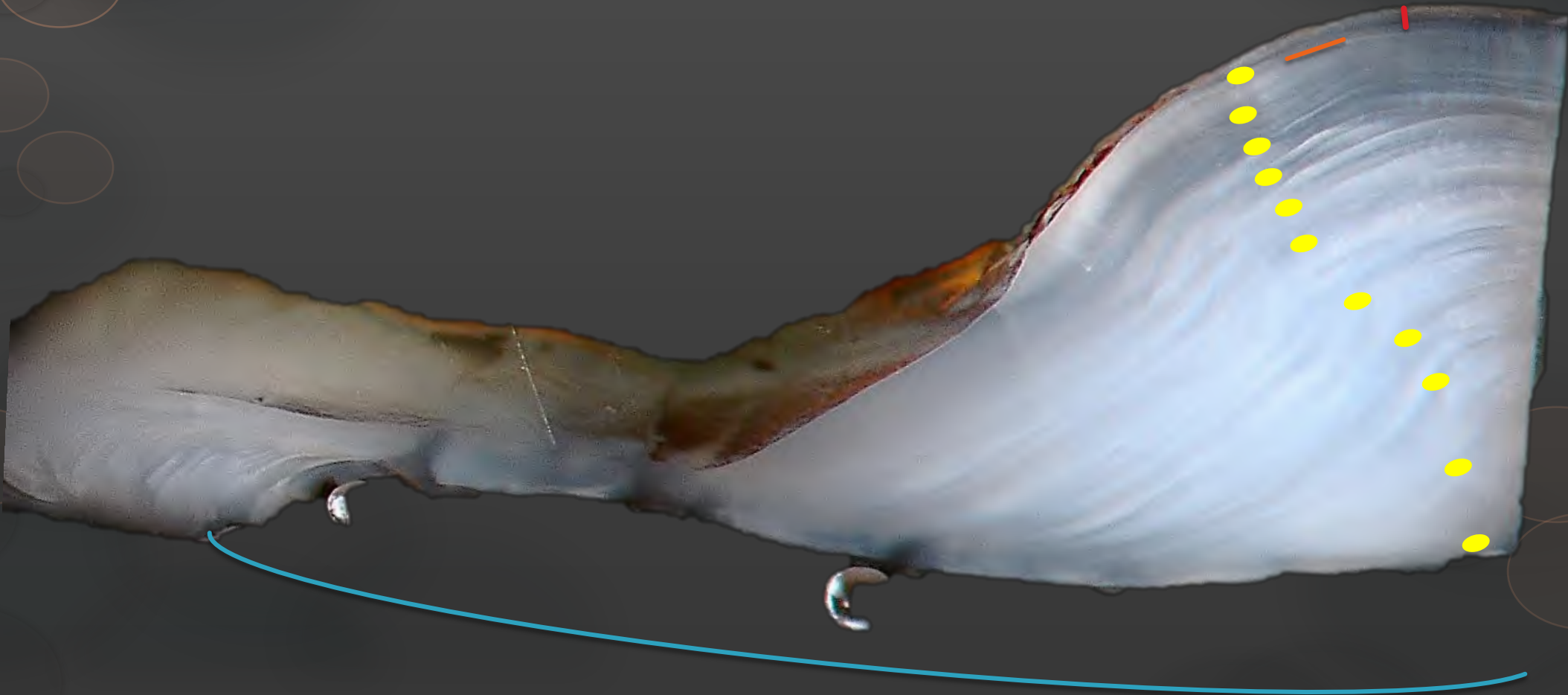
#6402 - Left Otolith Bulge Transverse Thin Section -
reflected light photo with growth indicator bar
transferred. Age estimate – 13.



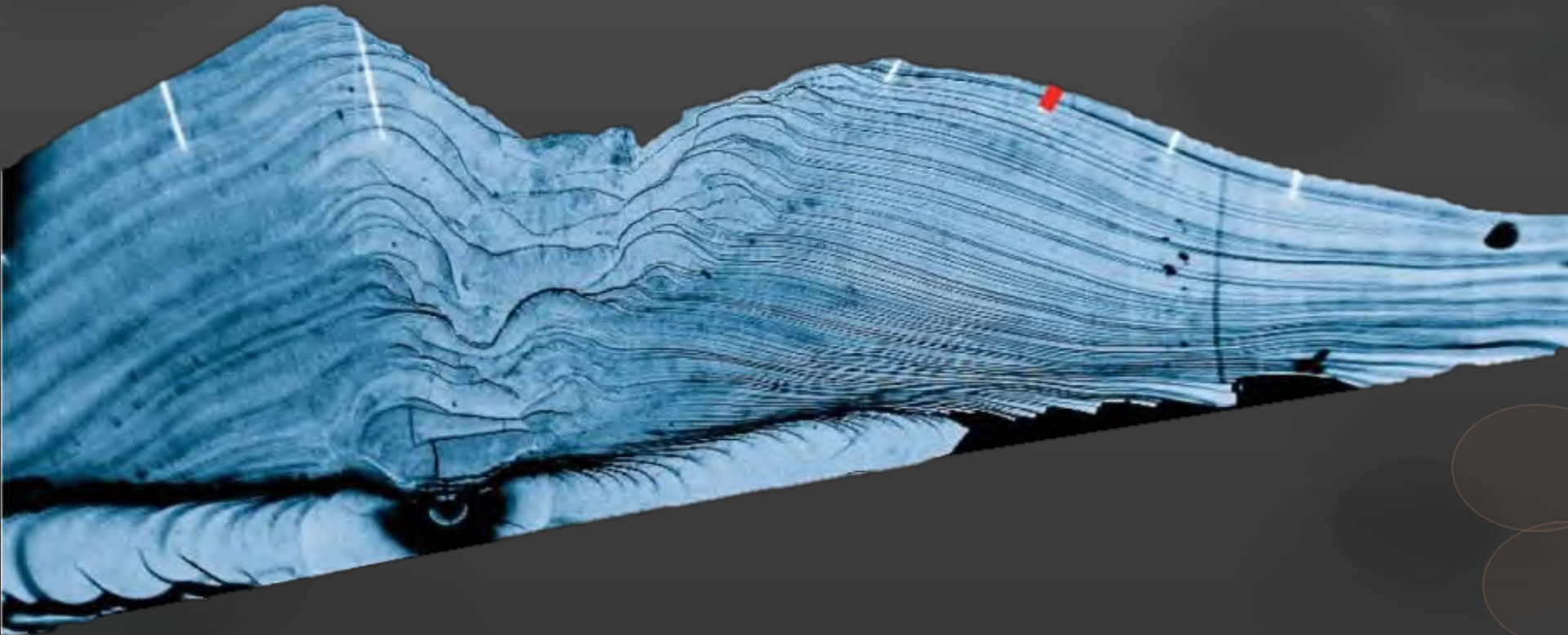
#6402 Left Otolith Longitudinal Thin
Section of the Remaining Posterior Part of
the Bulge. Back Scatter Electron Image
with a bar (red) indicating growth after the
SrCl mark.



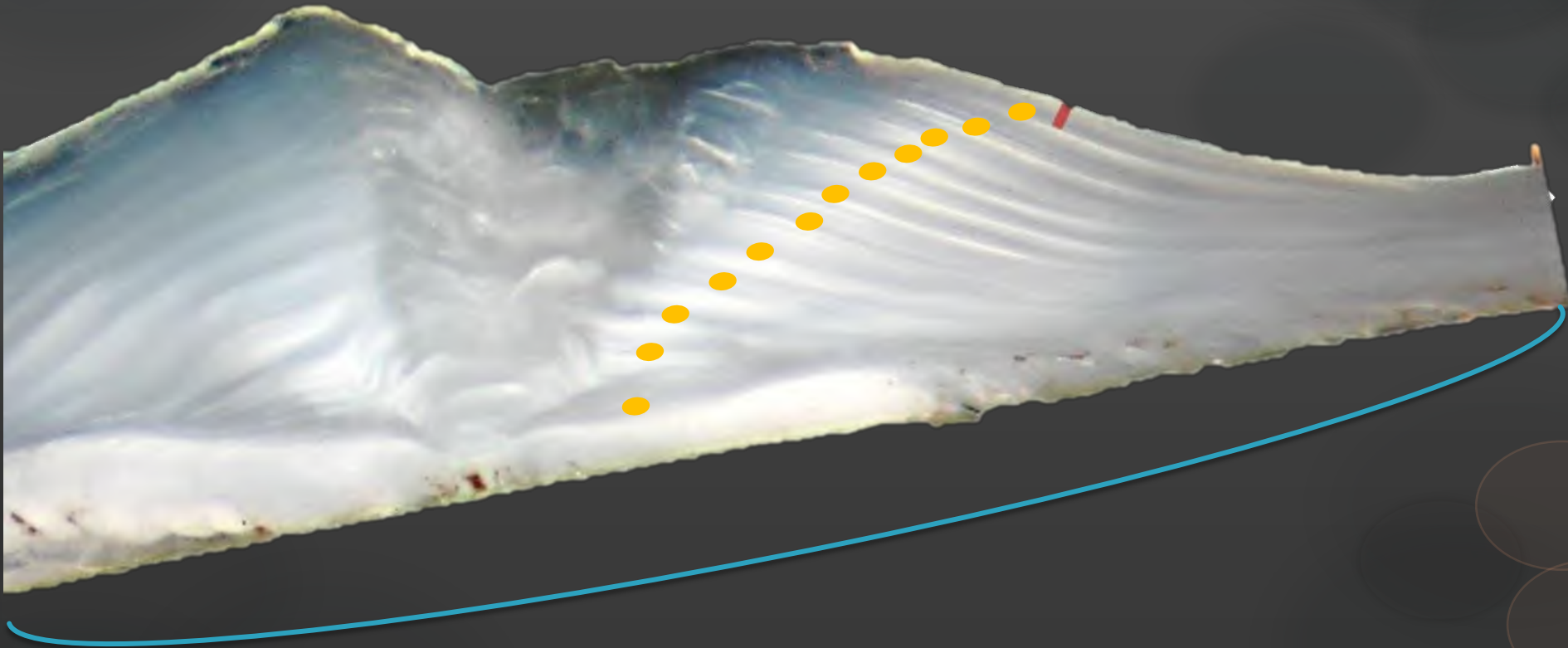
#6402 Left otolith longitudinal thin section of the remaining posterior part of the bulge. Reflected light photo with growth indicator bar (red) transferred. 11 annuli identified. Orange bar was inserted by me ahead of time to indicate where I thought the mark should be found based on the time at sea after marking.



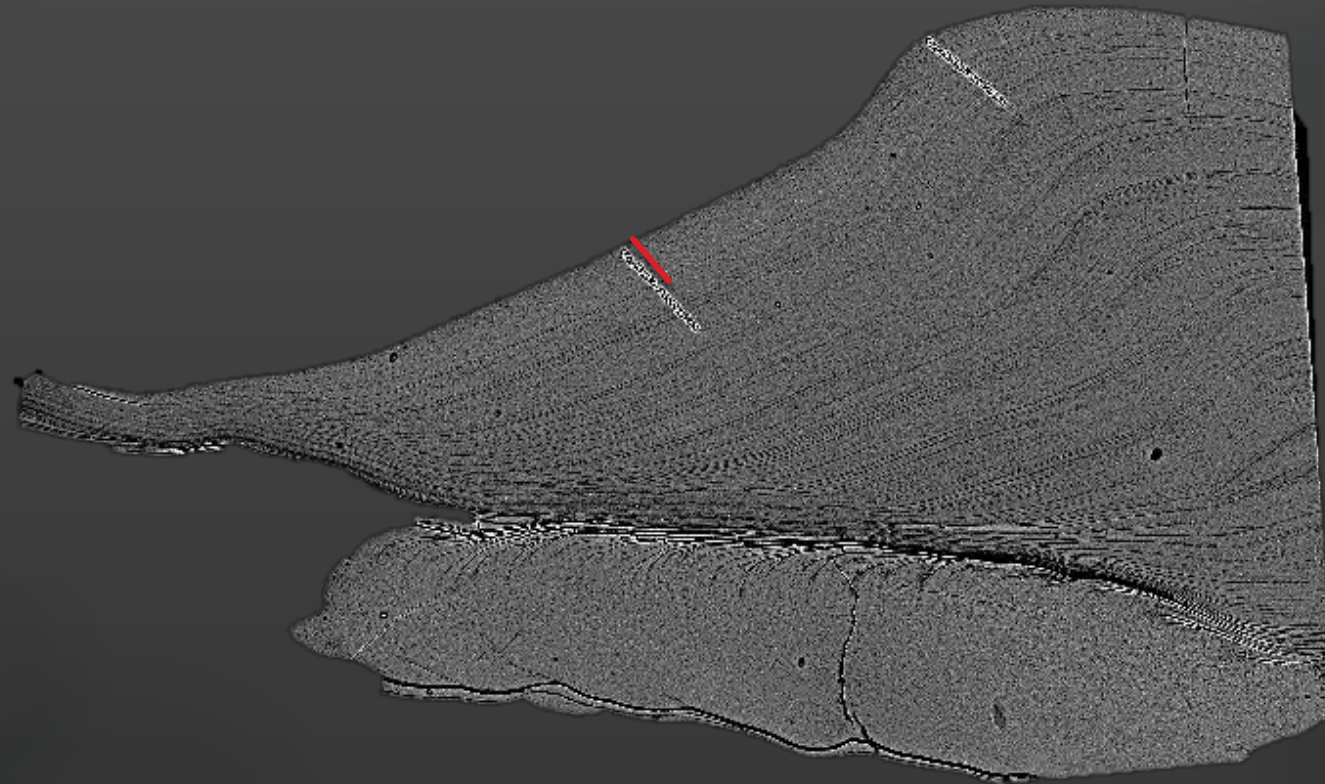
#538 (low dose) - Left Otolith Bulge Transverse Thin Section - Back Scatter Electron Image with a bar (red) indicating growth after the SrCl mark. Tagged Sept. 29, 2007 (low dose)(43 cm); recaptured Nov. 19, 2008 (46 cm). 13.9 months-at-large after marking; 3 cm growth.



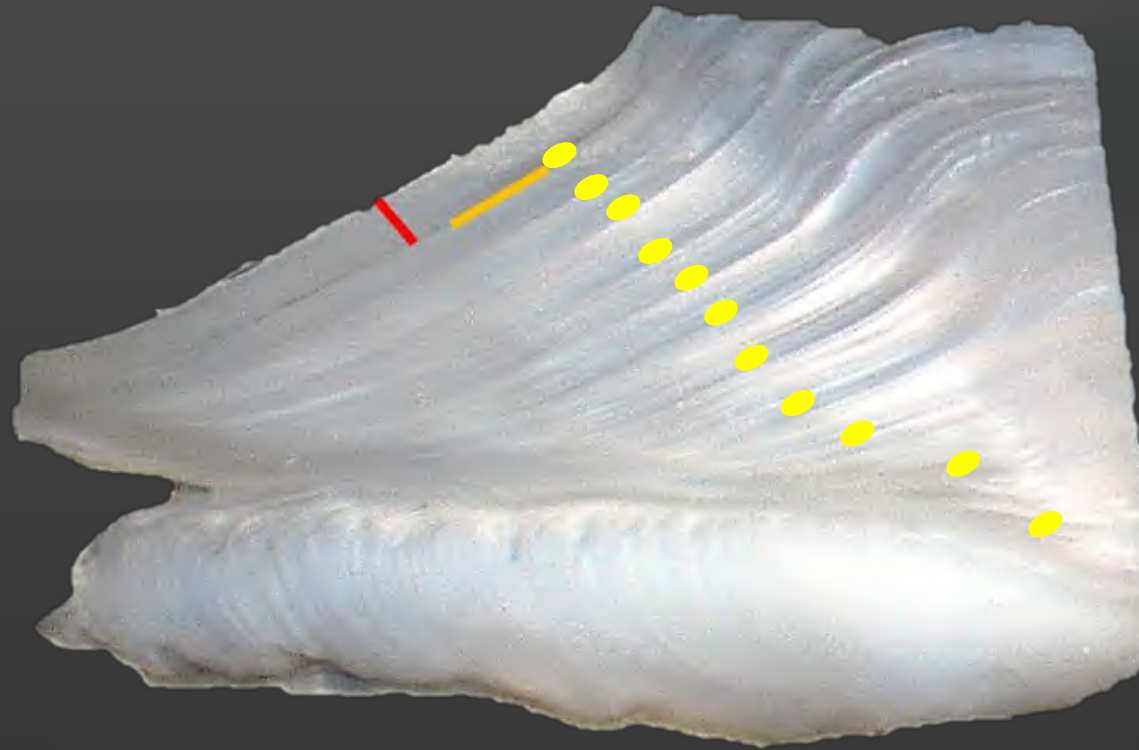
#538 Left Otolith Bulge Transverse Thin
Section - reflected light photo with growth
indicator bar transferred. Age estimate – 12+.



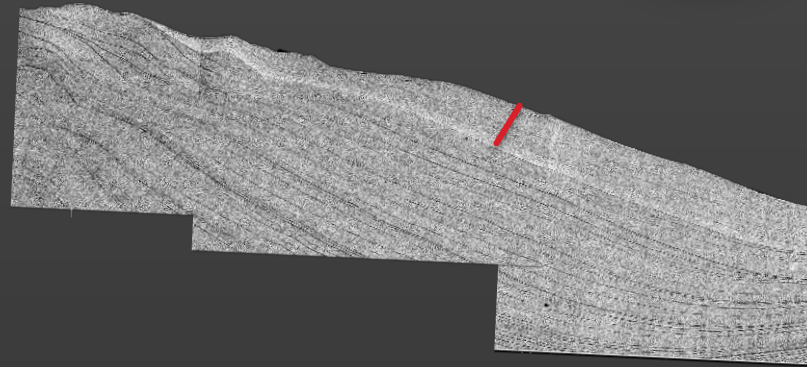
#538 Left Otolith Longitudinal Thin Section of the Remaining Posterior Part of the Bulge. Back Scatter Electron Image with a bar (red) indicating growth after the SrCl mark.



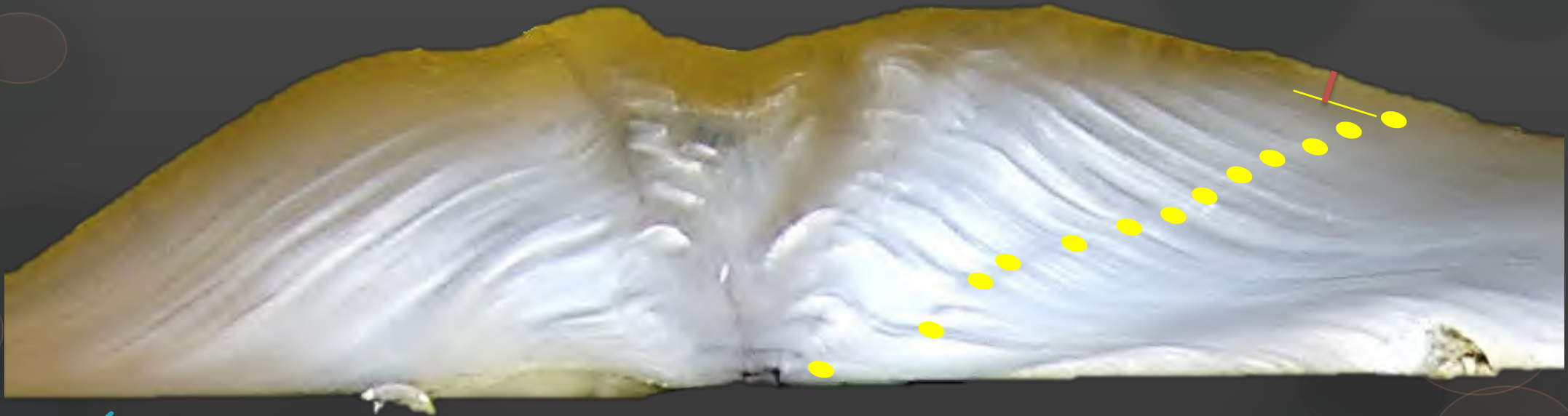
#538 Left otolith longitudinal thin section of the remaining posterior part of the bulge. Reflected light photo with growth indicator bar (red) transferred. 11 annuli identified. Orange bar was inserted by me ahead of time to indicate where I thought the mark should be found based on the time at sea after marking.



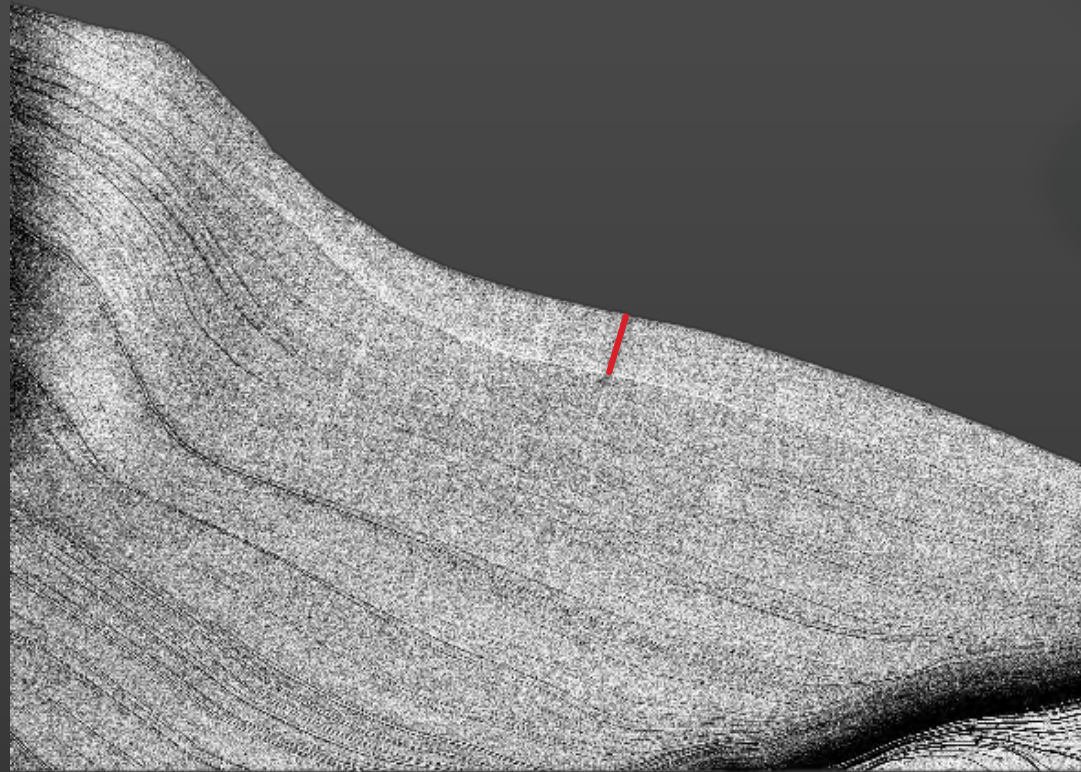
#0026 (low dose) - Left Otolith Bulge Transverse Thin Section - Back Scatter Electron Image (dorsal slope edge-mark visible) with a bar (red) indicating growth after the SrCl mark. Tagged Sept. 29, 2007 (low dose)(43 cm); recaptured Nov. 19, 2008 (46 cm). 13.9 months-at-large after marking; 3 cm growth.



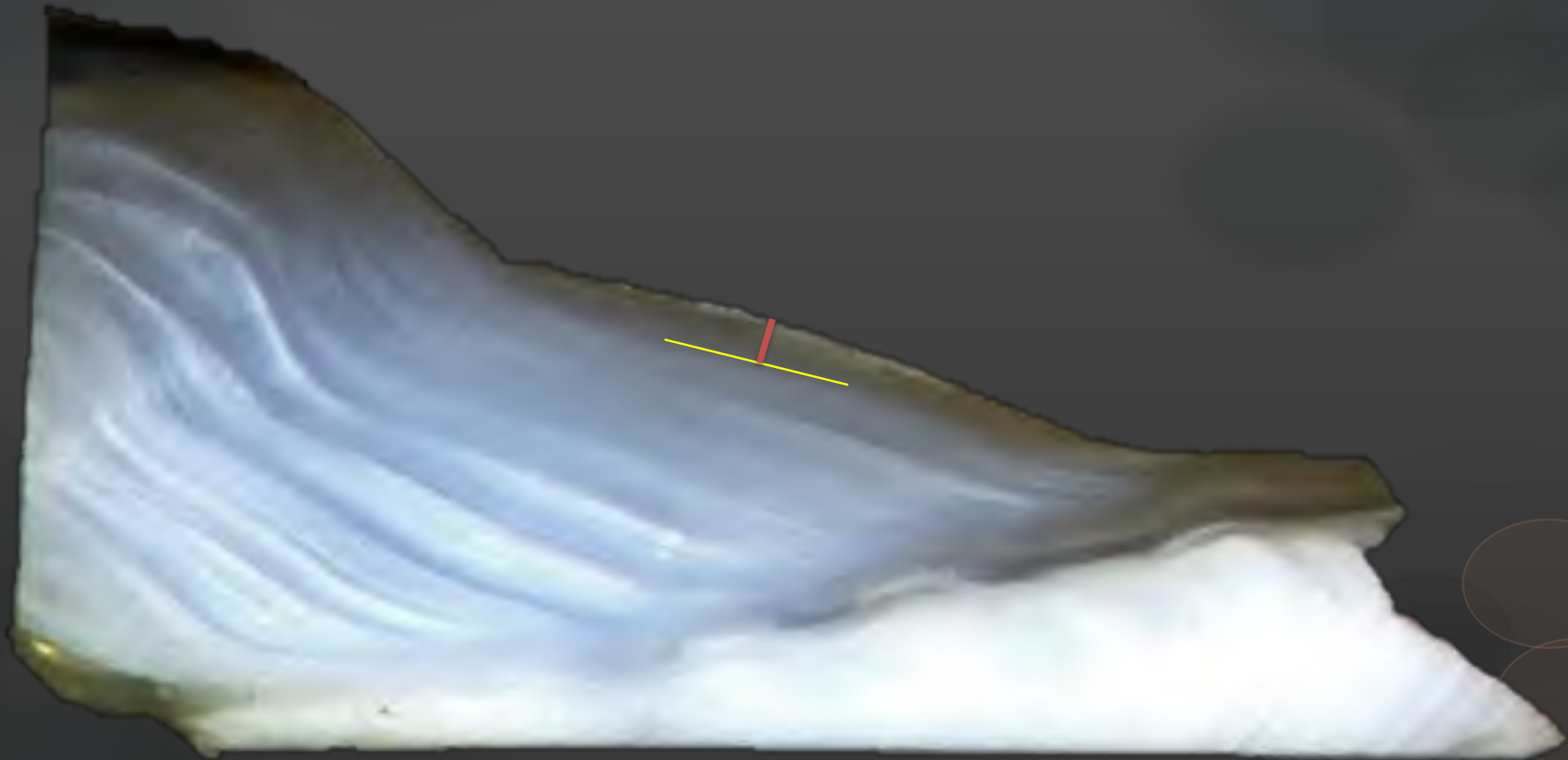
#0026 – Left otolith transverse thin section - reflected light photo with growth indicator bar transferred. (low dose; tagged Sept. 29, 2007, recaptured Nov. 22, 2008; 14 months out). Yellow bar was inserted by me ahead of time to indicate where I thought the mark should be found based on the time at sea after marking. Age estimate – 13+. [Portion below the 1st year annulus was ground away earlier when trying to find the mark on the whole otolith (lateral grind)].



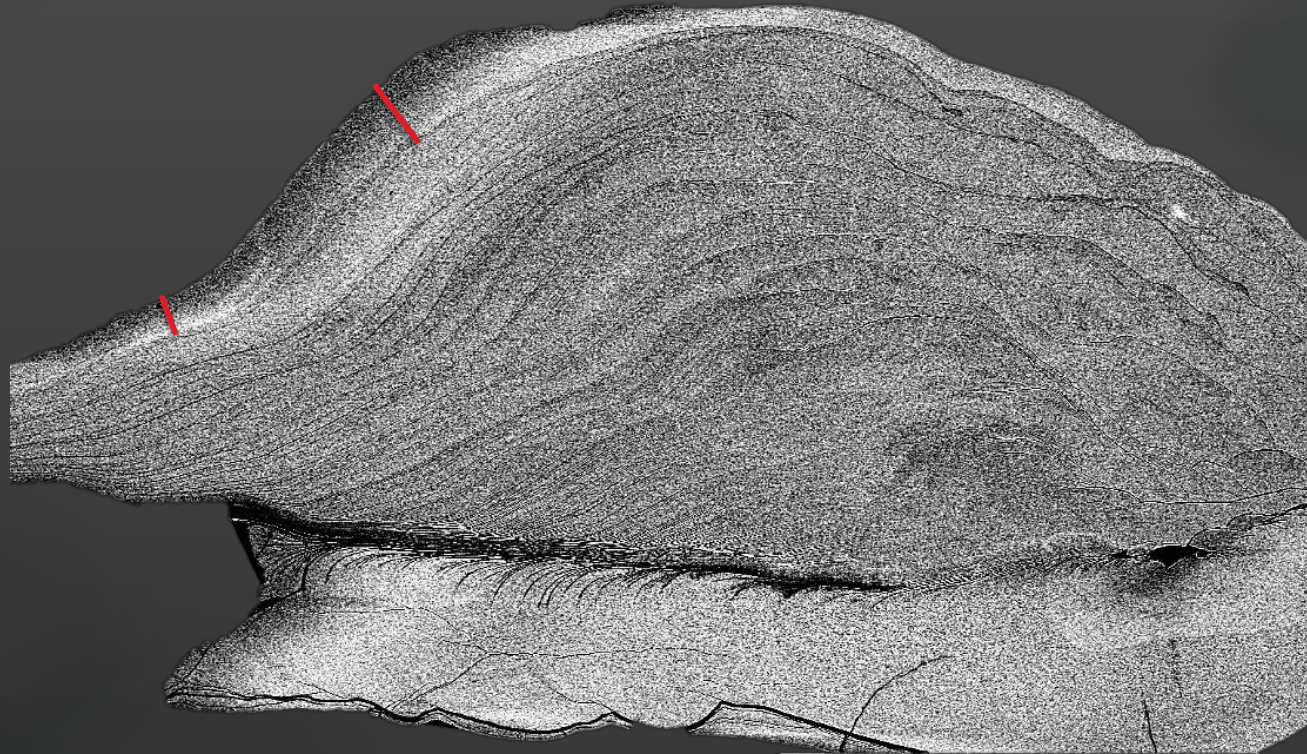
#0026 Left Otolith Longitudinal Thin Section of the Remaining Posterior Part of the Bulge. Back Scatter Electron Image with a bar (red) indicating growth after the SrCl mark.



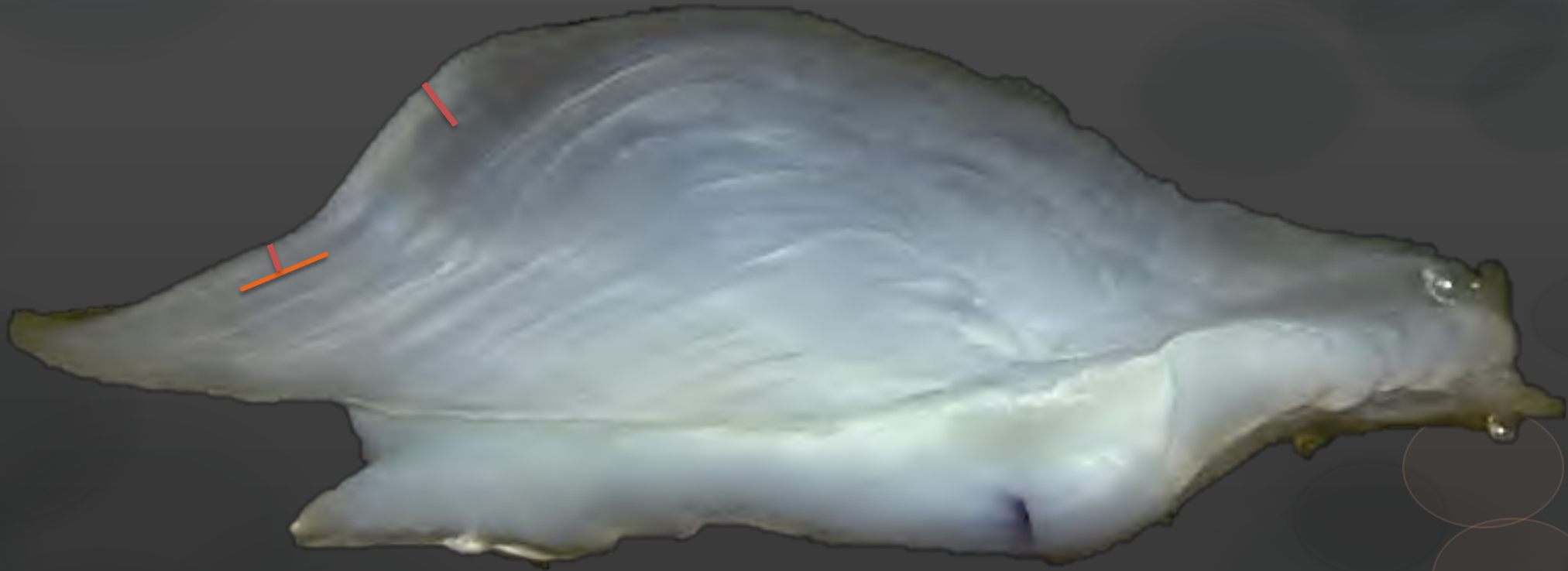
#0026 – Left otolith longitudinal thin section of the remaining posterior part of the bulge (reflected light). Reflected light photo with growth indicator bar (red) transferred. 10 annuli identified. (low dose; re-caught in November; 14 months out). Yellow bar indicates expected location of the mark.



#2823 (low dose) - Left Otolith Bulge Longitudinal Thin Section - Back Scatter Electron Image with a bars (red) indicating growth after the SrCl mark. Tagged Oct. 4, 2007(46 cm); recaptured Nov. 10, 2008 (46 cm). 13.4 months-at-large after marking.



#2823 Left otolith longitudinal thin section. Reflected light photo with growth indicator bars (red) transferred. 11 annuli identified. Orange bar was inserted by me ahead of time to indicate where I thought the mark should found be based on the time at sea after marking.



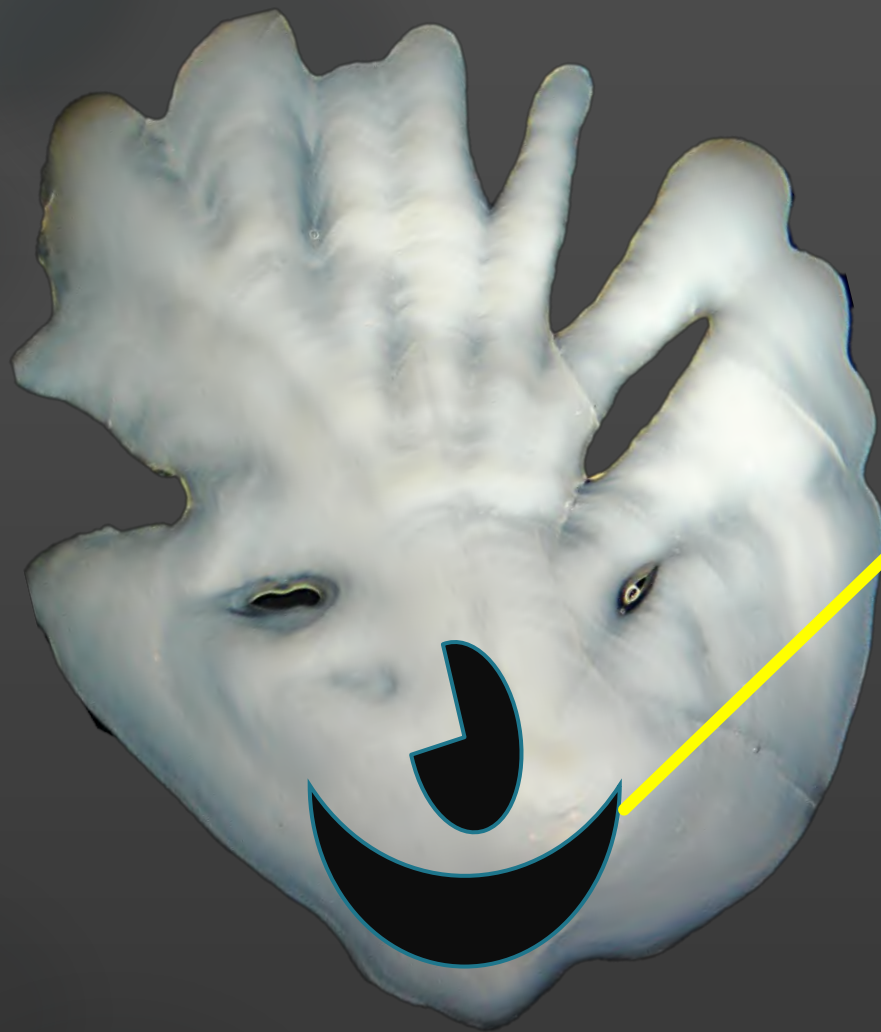
Conclusions

- There is some validation evidence supporting the sectioning of the left otolith bulge to estimate ages for Greenland halibut
- The longitudinal section plane has one significant advantage over the transverse – the nucleus and the bulge peak line up much more consistently on that plane
- Baking the otoliths before sectioning tends to reduce some of the confusion encountered when trying to distinguish between annuli and checks while attempting to read these sections

Future Directions of the FWI Otolith Lab

- Development of operating procedures for our lab.
- Procurement to access analytical machines at collaborative institutions
- Training manual for when senior technicians retire.
- Research and development for otoliths and other age structures.
- Building a network within and external to DFO on otolith based research.





Thank-You!